Study of the eyespot disease of cereals caused by *Pseudocercosporella* spp.

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To my parents

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ABSTRACT

The eyespot disease of cereals was found to be prevalent across Southland and also in areas of Otago and Canterbury. Surveys between 1984-1986 showed the disease was more severe with frequent lodging in the 1984/85 season. Wheat, barley, rye and triticale crops were sampled and isolates of *Pseudocercosporella* obtained. Isolates of fast-even morphology remained the predominant type. Slow-feathery isolates increased in proportion during this time and were not significantly higher on barley than wheat, as is the case in the U.K.

Electrophoretic analyses of esterases present in *Pseudocercosporella* provided evidence for the separation of fast-even and slow-feathery morphological types but not species. Variation in minor bands was detected between isolates from both within N.Z. and between N.Z. and Europe.

In vitro resistance to the fungicide benomyl, was found in the Southland *Pseudocercosporella* population at all tested concentrations, ranging from 0.2 - 200 ug a.i. ml^{-1}. Proportions of isolates resistant to 2 ug a.i. ml^{-1} remained similar over the sampled seasons. Isolates produced the same responses in shaking liquid-culture as they did on solid agar media. Resistance was detected in both sprayed and unsprayed crops.

Insensitivity to the demethylation-inhibiting (DMI) chemical, prochloraz, was detected at all tested concentrations ranging from 0.1 - 200 ug a.i. ml^{-1}. Both hyphal growth and sporulation was suppressed in only a proportion of subcultures from an isolate, however proportions of insensitive subcultures were consistent for each isolate. DMI-insensitive responses were numerous with small differences in EC50 values and occurred in isolates distributed widely within the wild population.

Negatively-correlated cross resistance between benomyl and MDPC was not found in *Pseudocercosporella*, hence MDPC would be of no use for controlling benomyl-resistant isolates.

The presence of acenaphthene did not induce its requirement in isolates of *Pseudocercosporella*.

A fungicide spraying trial undertaken in Southland demonstrated that in the presence of benomyl-resistance, prochloraz gave better eyespot control than benomyl. The use of mixtures and efficient spray application is discussed.

N.Z. slow-feathery and fast-even isolates of *Pseudocercosporella* were shown to have the expected association with R-type and W-type pathogenicities described of overseas isolates. Wheat cultivars screened for response to eyespot were highly susceptible except for Bounty, which contains Capelle-Desprez resistance, and Takahe. The barley cultivars were also susceptible to both fungal pathotypes, and of the rye cultivars, Dominant was the most resistant. The triticale cultivars had varying responses, with Karere being susceptible to both pathotypes, Lasko susceptible to R-types and Salvo and Aranui moderately resistant to both pathotypes. A range of scores was obtained for wheat breeding lines which were screened, but all were quite susceptible. A source of resistance was identified in a line of *Hordeum bulbosum* L.

Epidemiology trials undertaken in Southland and Canterbury showed a dependence of inoculum dispersal on rain-bearing wind. Fast-even isolates appeared to spread more quickly, however slow-feathery isolates became better established in plots inoculated with isolates of both morphological types. The natural infection time is suggested to be late winter/early spring in Canterbury. An unsuccessful attempt was made to detect a perfect state of the fungus on stubble and to induce it in culture.

A trial evaluating yield in barley following eyespot infection was undertaken in Southland and a significant reduction in head weight was obtained.

1.0 GENERAL INTRODUCTION

1.1 EYESPOT DISEASE

Eyespot disease common in cereals and many grasses, is caused by a complex of *Pseudocercosporella* species, *P. herpotrichoides* var. *herpotrichoides*, *P. herpotrichoides* var. *acuformis*, *P. anguioides* and *P. aestiva* (Nirenberg, 1981). The disease is recognized by characteristic symptoms on the basal haulms of hosts, not usually above the third node. Infections result in slow-forming lesions which begin as small stem discolourations and extend longitudinally and transversely through the stem. Lesions consist of pale, oval spots with darkened margins (Fig. 1.1). The stem lumen is commonly filled with mycelia of white to dark-grey colouration. At the point of infection, stems weaken and often lodge, eyespot lodging being characterised by a diagonal bend or 'kink'. Maturing crops often produce shrivelled grain and partially empty ears, even if lodging does not occur (Sprague and Fellows, 1934), owing to the inhibition of nutrient transport within the damaged stem. Eyespot has also been referred to as 'Pietin' (Foex, 1919; Foex and Rosella, 1930) 'black foot of cereals' (Fron, 1912), 'footrot of cereals' (Heald, 1920; Sprague and Fellows, 1934; Nirenberg, 1981), 'Columbia Basin Footrot' (Sprague, 1931) and 'strawbreaker' (Bruehl *et al.*, 1968). Similar symptoms may be caused by numerous other fungi, including *Rhizoctonia solani* Kuhn, *Ceratobasidium cornigerum* (Bourd.) Rogers and *Waitea circinata* Warcup & Talbot which are responsible for the 'sharp eyespot' disease of cereals and *Gaeumannomyces graminis* (Sacc.) Arx & Oliv. which causes 'take-all' disease. *Gibberella avenacea* Cook, *G. fujikuroi* (Saw.) Wr., *Micronectriella nivalis* (Fr.) Ces, Rabenh. and *Fusarium culmorum* (Smith) Sacc. commonly cause foot rot along with darkening of the basal haulms. *Pseudocercosporella* may often occur in mixed infections with these fungi. It is important to be able to distinguish the causal agents since sharp eyespot, for example, declines at continuously-cropped sites, whereas eyespot builds up (Glynne, 1972). Eyespot symptoms have been described from as early as 1878 (Fron, 1912) in France, although the causal organism was not known at that time. Eyespot was first recorded in N.Z. in 1943 (Saxby, 1943).

1.2 HOSTS

Hosts of the eyespot fungus include species of *Triticum, Hordeum, Secale, Avena, Aegilops, Agropyron, Bromus, Poa, Dactylis, Lolium, Agrostis, Alopecurus, Festuca, Koeleria* and Triticosecale (Sprague, 1936; Booth and Waller, 1973).

1.3 DISTRIBUTION

Eyespot disease is widespread, with reports of *Pseudocercosporella* occurring in America, Europe, Asia, Australasia and Africa (Booth and Waller, 1973).

1.4 TAXONOMY AND NOMENCLATURE

There has been confusion in the literature for many years concerning the taxonomy of the eyespot fungus and changes are still being made. On the basis of conidial type, Fron (1912) proposed the name *Cercosporella herpotrichoides* to describe the eyespot fungus, which he held to be the imperfect stage of *Leptosphaeria herpotrichoides* de Not. Foex (1919), however, claimed that *Cercosporella* and *Leptosphaeria* were not genetically connected and in 1930 Foex and Rosella described the cause of "le champignon des taches ocellees" as "champignon x". A footrot of cereals was described in Washington, U.S.A. (Dana, 1919) and either *Rhizoctonia solani* or *Ophiobolus graminis* Sacc. (syn. *Gaeumannomyces graminis* (Mont.) de Bary) was suggested as the cause. The stem lesions were similar to those caused by *Rhizoctonia solani*, but hyphal diameters were only about half as large. Sprague (1931) studied the eyespot fungus in Washington and Oregon and concluded that it was taxonomically identical with *C. herpotrichoides* and gave an amended description. Sprague and Fellows (1934) described the conidia of *C. herpotrichoides* as being 2-several (mostly 5-7) septate and measuring 30-80 (mostly 40-60) by 1.5-3.5u with conidiophores being sometimes branched. This extended Fron's original description of 3-septate conidia measuring 32-38 by 1.5-2.0u. Deighton (1973) transferred the genus to *Pseudocercosporella*, following study of a culture isolated from *Agropyron repens* (L.) Beauv. Details of conidiogenous cells were described :

The ends of branched hyphae "developed into almost colourless sympodial conidiophores up to 20u long and 3-3.5u wide with 2 or 3 rather distant septa. Occasionally a small ellipsoid conidiogenous cell was borne as a lateral branch of a mycelial hypha. Conidial scars truncate, unthickened and inconspicuous, about 1u diameter. Conidia colourless, acicular, widest at about a third of a length from the base, with a truncate unthickened hilum, straight or very slightly curved, smooth, 3-7 septate not constricted, 26.5-17 x 1-7u.".

The fungus was thus removed from *Cercosporella* and placed in *Pseudocercosporella*, of which it was the only species yet known in culture.

In 1981, the species was further subdivided and two new species described. On the basis of morphological characteristics (Table 1.1), namely conidial size and shape, Nirenberg (1981) differentiated the species into:-

P. herpotrichoides Fron (Deighton) var. *herpotrichoides*,
P. herpotrichoides Fron (Deighton) var. *acuformis* Nirenberg,
P. anguioides Nirenberg and
P. aestiva Nirenberg.

In 1984, Nirenberg, using a range of physiological tests, substantiated the independence of these species. Anastomosis was observed only between strains of the same taxon. Antagonism tests showed that of all *Pseudocercosporella* isolates, only isolates of the two varieties of *P. herpotrichoides* were able to markedly inhibit the growth of *Rhizoctonia cerealis* Van der Hoeven and *Aureobasidium pullulans* (de Bary) Arnaud. Agar modified with 3% fructose reduced hyphal growth of the two *P. herpotrichoides* varieties and completely inhibited *P. anguioides*. Glucose, saccharose and maltose had no significant effects on any of the three species. It was suggested that *P. herpotrichoides* var. *acuformis* had the highest carbendazim sensitivity. Temperature had varying effects on the different taxa. At 15°C *P. herpotrichoides* var. *herpotrichoides* failed to sporulate at all. Differences in regional and seasonal occurrence of the species were noted (Nirenberg, 1985) and hence it was suggested that

optimal time for applying chemical control could vary. Numbers of plants found infected with *P. herpotrichoides* var. *acuformis*, *P. herpotrichoides* var. *herpotrichoides*, *P. anguioides* and *P. aestiva* declined in that respective order.

Recently a teleomorph of the fungus was described in Australia (Wallwork H., *pers. comm.*). Straw of wheat, *Bromus diandrus* Roth. and *Hordeum leporinum* Link bearing eyespot lesions, were placed on moist sand in petri dishes and incubated at 10°C. After about three months apothecia formed on the straw and resembled those produced by the genus *Tapesia*, an ascomycete in the family Hyaloscyphaceae. This recent finding of a sexual state will aid elucidation of the correct taxonomic status of the fungus.

Table 1.1 The species as described by Nirenberg, (1981).

SPECIES	SEPTA	CONIDIAL SIZE	COLONY DIAMETER AT 20°C AFTER 10 DAYS
P. herpotrichoides var. *herpotrichoides*	mostly 4-septate	35.0- 80.0 (51.7) X 1.5-2.5 u straight and curved	12mm
P. herpotrichoides var. *acuformis*	4-6 septate	43.0-120.0 (65.8) X 1.2-1.3 u	5mm
P. anguioides	6-8 septate	80.0-260.0 (152.0) X 1.0-1.5 u	11mm
P. aestiva	3 septate	15.0- 32.0 (23.6) X 1.0-1.5 u	6mm

1.5 PATHOGENIC VARIATION

Isolates of *Pseudocercosporella* have been divided into distinct categories, based on morphological, physiological and pathogenic variation. In 1952, Glynne reported an isolate from rye being more virulent on rye than on wheat or barley. Lange-de la Camp (1966) stated that although no *formae speciales* exist, there are differences in aggressiveness. Isolates were divided into wheat-type (W-type) and rye-type (R-type) categories. R-type isolates are equally pathogenic to wheat and rye and W-type isolates are less pathogenic to rye than wheat, however, both high and low aggressiveness has been found within each type.

During a series of experiments, although pathogenicity of isolates varied, the relative order of host susceptibilities remained the same. Wheat was usually the most severely infected, with barley less and oats and rye the least (Scott *et al.*, 1975).

In comparison with W-type isolates, which have an 'even' colony edge, R-types grow more slowly *in vitro*, have a 'feathery' colony edge and limited development of aerial mycelium. R-type isolates produce more pigmentation on the underside of colonies and in the agar, sometimes exhibit a slimy growth habit and often sporulate more readily. W and R-type isolates have become synonymous with fast-even (FE) and slow-feathery (SF) types. In the U.K., R-type isolates are more common than W-types on barley and pose a serious threat to barley, triticale and rye (Scott and Hollins, 1985). King and Griffin (1985) suggest it is likely that W-type and R-type correspond respectively to *P. herpotrichoides* var. *herpotrichoides* and *P. herpotrichoides* var. *acuformis*. For convenience in this study, the name '*Pseudocercosporella*' is used and isolates identified as either fast-even (FE) or slow-feathery (SF).

1.6 FACTORS AFFECTING THE DEVELOPMENT OF EYESPOT

Primary infection is by mycelium and conidia originating from crop stubble or alternate hosts, however ascospores must also play a role. As the perfect state has only recently been found, its importance in

primary infection is not yet known. Conidia are produced from crop debris when conditions are cool and moist and these are then spread, generally by rain-splash (Glynne, 1953; Ponchet, 1959; Rowe and Powelson, 1973a), usually in large ballistic splash droplets although sometimes in smaller airborne droplets (Fitt and Bainbridge, 1983). Dispersal of conidia by dry, strong winds has been demonstrated (Ponchet, 1959; Fehrmann and Schrodter, 1971). Conidia have been found in soil suspensions (Dickens, 1964) and in water that has been passed over infected debris (Jordan and Tarr, 1978). It was suggested by Diercks, (1965), that infection can occur via the soil. Inoculum for establishing secondary infection originates from sporulating primary lesions, however Rowe and Powelson (1973b) determined the effective dispersal range of *Pseudocercosporella* conidia to be only 1-1.5m and described the disease as 'simple interest' *sensu* Vanderplank, in which the inoculum source is fixed and increase in disease with time is arithmetic. *Pseudocercosporella* was described as being unable to produce significant amounts of secondary inoculum to be a compound disease. Tillage and wind-blown infected debris were considered the probable inoculum capable of long-range dispersal.

Following studies of *in vitro* nutrient requirements and comparative straw colonisations with other fungi, *Pseudocercosporella* was found to have a limited competitive saprophytic ability and hence thought unlikely to colonise straw in soil (Macer, 1961a). Straw artificially colonised with *Pseudocercosporella* before being buried in soil decomposed more slowly than uncolonised straw and this has been suggested to provide a means for the fungus to survive for long periods. The maximum time for which test straw was kept buried was three years, after which the fungus was found to have survived and remained 'virtually undiminished in vitality', with 76% of the straws still capable of sporulating. Survival was not as good on straws kept close to or on the surface of the soil. Sporulation declined in straws kept at a depth of 2.5cm for more than 79 weeks and straws kept on the soil surface had reduced sporulation after only 34 weeks (Macer, 1961b). Work with naturally-infected straws (Cox and Cock, 1962), suggested that one year without a cereal host would be too short to reduce *Pseudocercosporella* to an insignificant level. Byther

and Powelson (1966) found the fungus capable of germination, growth and sporulation within field soil, showing at least short-term saprophytic survival in soil to be likely.

Disease incidence has been positively correlated with the number of wet days per week (Van der Spek, 1975; Jordan and Tarr, 1978; Hollins and Scott, 1980). Differences in infection severity have been noted between winter and spring-sown cereals and thought more likely to be caused by the delay in crop development rather than the delayed exposure to inoculum, however, both factors probably play a role to some extent (Hollins and Scott, 1980).

Fitt (1985) considered the stages of development of eyespot lesions in wheat plants, following initial infection, to be:- a) leaf sheath penetration, b) stem lesion establishment (growth of the fungus from leaf sheaths into the stem) and c) stem lesion development. The rate of leaf sheath penetration was found to decrease when conditions were cold and dry, partly as a result of the slower formation of new leaf sheaths. Small changes in temperature and relative humidity did not greatly affect penetration. Lesion establishment was better related to accumulated temperatures than to time or accumulated rainfall and is only a short-term stage. The crucial stage at which lesions become established depends upon the time the basal leaf sheaths remain on the stem after the stem has elongated, and on the rate at which the fungus colonises the stem from the infected leaf sheath.

Scott (1971) found that the number of leaf sheaths penetrated by the fungus increased with temperature. In growth room experiments this was not linear, but in different temperature regimes the rate of penetration was approximately uniform after an initial lag phase. As these responses to temperature parallel those of the fungus growing on agar, it is probable that effects of temperature on the pathogen alone rather than on host susceptibility, could be responsible for increased host penetration.

With vegetative mycelium successfully persisting in ground debris for long periods of time, control of the fungus can be made difficult. Crop-sequence planning, which is the traditional method for

controlling any soil-borne disease, is effective if a non-cereal crop is planted between cereal crops to allow inoculum to decline. A one-year break from cereals, however, may still allow subsequent infection by dormant propagules in crop debris. Burning of stubble effectively decreases the proportion of infected straw, but will not completely eliminate the fungus as the presence of only small patches of green weeds are enough to prevent small pockets of stubble from flaming. As *Pseudocercosporella* has wild grass hosts, such weeds may themselves maintain the fungus, highlighting the importance of crop hygiene. Slope and Etheridge, (1970) found that flaming before ploughing slightly decreased the occurrence of eyespot, however, burning after ploughing did not. Fig. 1.2 shows the success of flaming in reducing severity of subsequent infection. The lodged strip around the outside of the field is an area which was ploughed and left as a firebreak in the previous season. Subsequent eyespot infection in the firebreak was severe and the plants lodged. Infection was also present in the main area of the field, but lesions were minor, hence lodging did not occur.

1.7 IMPORTANCE OF EYESPOT DISEASE IN N.Z. AND AIMS OF THE CURRENT STUDY

In N.Z., eyespot is chiefly important on wheat and barley in Southland, where high rainfall and the growing of susceptible varieties promote infection. It also occurs in Otago (Saxby, 1943) and some regions of Canterbury.

Chemical control of eyespot is effective in N.Z., hence host resistance has not been a high priority in breeding programmes. Early control measures consisted of good crop management and the use of straw-shortening chemicals to help prevent lodging. Protectant fungicides such as calcium cyanamide and sulphuric acid were used. In the early 1970s, the systemic fungicide benomyl was introduced commercially in N.Z. and control has been successful, although *Pseudocercosporella* isolates resistant to the chemical were detected in 1984 (King *et al.*, 1984). No problems with field control have been reported, unlike control of European eyespot populations, in which

high proportions of benomyl-resistance have developed (Griffin *et al.*, 1983; Brown *et al.*, 1984). In 1985, prochloraz (Sportak 45EC, Schering Ltd) was also registered in N.Z. for eyespot control and some growers are now using this instead of, or alongside, benomyl. In the U.K., in response to the resistance problem, benomyl is now only available in combination with prochloraz for eyespot control (commercially known as 'Sportak Alpha'). This product is not available in N.Z. as it is too expensive to be competitive.

There are many opponents to the application of chemicals to crops, with concern over residues in harvested grain and general environmental pollution. An increasing development of fungicide resistance means chemical control may be less durable. The growing of resistant cereal cultivars must provide the most acceptable form of control.

Much work has been done studying responses of *Triticum*, *Aegilops*, *Secale*, and *Hordeum* species and cultivars to infection by *Pseudocercosporella* in the hope of finding a source of resistance. Durable resistance to eyespot is exhibited by the French cultivar, Capelle-Desprez, and this has been introduced into many European cultivars such as Maris Huntsman and Bounty (Ingle *et al.*, 1980). The genetic nature of this resistance has not been characterised and it is thought unlikely that superior resistance could be gained by its incorporation into suitable crossing programmes (Doussinault and Dosba, 1977). One high-level resistance gene has been identified in *Aegilops ventricosa* Tausch (Doussinault *et al.*, 1983) and has been incorporated into cultivars, such as Rendezvous in England (Sanderson, *pers. comm.*). The incorporation of single resistance genes into new cultivars must be treated with caution, however, as there is a higher likelihood of the resistance breaking down, as was the case with the directional selection of *Phytophthora infestans* (Mont.) de Bary described by Vanderplank in 1978.

As *Pseudocercosporella* adapts within local populations, it is important to have a knowledge of the gene pool of the population in the area for which new cultivars are being bred. Cunningham (1981) in the U.K. stated that "The initiation of cereal breeding programmes for

resistance to *P. herpotrichoides* (Fron) Deighton, has created the need for thorough evaluation of pathogenic variation in the fungus.". Disease nurseries in N.Z. have concentrated on cultivars of *Triticum*. In the last few years, however, there has been an upturn in the export market for malting barley, and triticale has become commercially-available, and hence the number of hectares sown in wheat and rye has declined. The need developed for an investigation of variation in pathogenicity of N.Z. isolates on N.Z. cultivars of these species.

AIMS OF THIS STUDY WERE TO:-

1. Determine the occurrence and distribution of *Pseudocercosporella* within Southland, N.Z., and to compare morphological, biochemical and growth attributes of local isolates with findings overseas.

2. Study responses of different isolates of *Pseudocercosporella* to the fungicides benomyl and prochloraz, which belong to different chemical groups and have different modes of action.

3. Evaluate field aspects of chemical control of eyespot.

4. Determine variation in pathogenicity of selected N.Z. isolates of *Pseudocercosporella* to N.Z. cultivars and breeding lines.

5. Assess the relative spread and build-up of isolates of different *Pseudocercosporella* pathotypes at a site over two seasons.

6. Assess the effects of different levels of eyespot inoculum on yield in barley.

7. Investigate the presence of a perfect state.

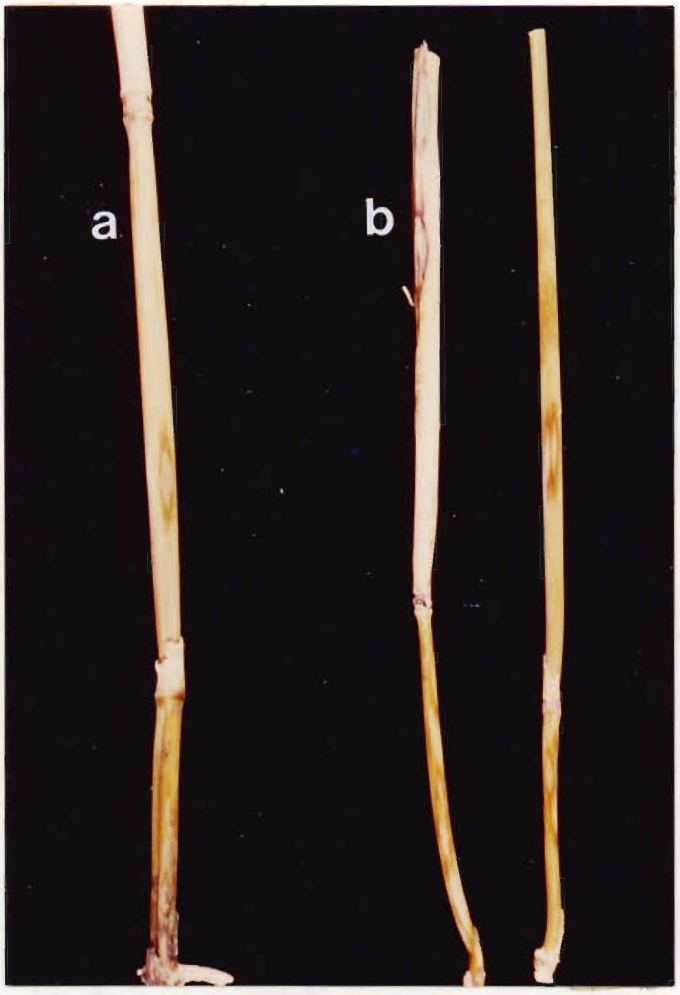


Fig. 1.1 Eyespot infected (a) triticale stem and (b) barley stems

Fig. 1.2 A crop of barley in Kelso, Southland, following a previous cereal burnoff. The lodged strip was a ploughed fire-break, allowing eyespot inoculum to build up.

2.0 METHODS AND MATERIALS

2.1 *IN VITRO*

2.1.1 Fungi

Isolates of *Pseudocercosporella* were obtained from cereal samples collected in Southland, Central Otago and Canterbury, NZ, during 1984-86. Some isolates were obtained from *ad hoc* commercial fungicide trials. English isolates were provided by Dr P.R. Scott, Plant Breeding Institute, Cambridge, England and isolates were also obtained from dried cereal stems collected in England and Germany by Dr F.R. Sanderson, Crop Research Division, DSIR, Lincoln. Isolates of *Rhynchosporium secalis* (Oudem) J.J. Davis were provided by Dr M.G. Cromey, Plant Diseases Division, DSIR, Lincoln. German isolates were also obtained from Dr H. Nirenberg. Australian isolates were provided by Mr J. Harris, CSIRO Division of Soils, Glen Osmond, South Australia, and Dr H. Wallwork, Plant Pathology Department, Waite Agricultural Research Institute, Glen Osmond, South Australia.

2.1.2 CHEMICALS

The following products were used in the study:

Acenaphthene	- British Drug Company
Agar	- Davis Gelatine (N.Z.) Ltd
Benlate	- Du Pont (New Zealand) Ltd
Citowett	- BASF NZ, Ltd
DPX H6573	- Du Pont (New Zealand) Ltd
Ethanol	- May and Baker Ltd
D(+)-Glucose	- Sigma Chemical Company, U.S.A.
MDPC	- ICI (New Zealand) Ltd
Sportak	- FERNZ Ltd
Streptomycin sulphate	- Sigma Chemical Company
WH bleach (5% sodium hypochlorite)	- Wilson Products (CHCH) Ltd

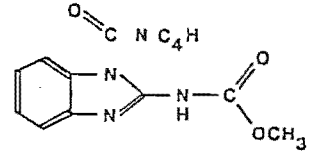
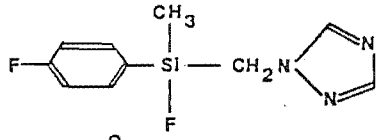
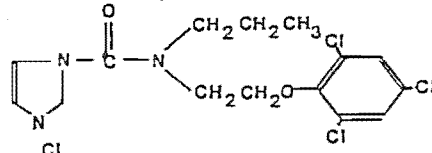
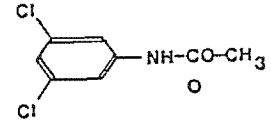
Chemical names, structures and formulations of the fungicides are listed in Table 2.1.

The following chemicals were used in the electrophoresis study and are all available from Sigma Chemical Company, U.S.A.:-

acrylamide, BIS (N,N - methylenebisacrylamide), bromophenol blue, disodiumphosphate, fast-garnet, glycine, monosodiumphosphate, ∝-napthyl-acetate, riboflavin, sucrose, TEMED (N,N,N,N - tetramethyl-ethylenediamine) and TRIS (Tris(hydroxymethyl)aminomethane),

Stock solutions of fungicides were freshly prepared each time they were required. Ethanol (analytical grade) was used to dissolve water-insoluble powders, and solutions were made using sterile, distilled water.

Table 2.1 Fungicide names and formulations

COMMON NAME	TRADE NAME OR CODE	FORMULATION	CHEMICAL NAME	STRUCTURAL FORMULAE
Benomyl	Benlate Bavistin	WP** 50%	methyl 1-(butyl carbamoyl) benzimidazole 2 yl carbamate	
TRIAZOLE	DPX H6573	DF** 20%	bis (4-fluorophenyl)methyl (1H-1,2,4-triazol-1-yl methyl) silane	
Prochloraz	Sportak	EC**45%	1-(N-propyl-N-(2-(2,4,6-(trichlorophenoxy)ethyl) carbamoyl) imidazole	
MDPC	MDPC	WP 25%	methyl N-(3,5-dichloro phenyl)-carbamate	

** WP = wettable powder, DF = dry flowable, EC = emulsifiable concentrate

2.1.3 Culture media

Potato dextrose agar (PDA) was used as a solid, complete medium for fungal growth. Each litre consisted of a filtered solution prepared by double boiling 300g potatoes in distilled water, supplemented with 20g d-glucose and 15g agar.

PDA was supplemented with 100ug ml^{-1} streptomycin sulphate to inhibit bacterial and fungal contamination during isolations from plant material.

Wheat extract dextrose agar (WEDA) and barley extract dextrose agar (BEDA) were as for PDA with double-boiled wheat or barley straw replacing the potatoes.

Wheat dextrose agar (WDA) and barley dextrose agar (BDA) consisted of PDA poured into sterile petri dishes containing sterilised wheat or barley straw cut into 25mm lengths.

Distilled water agar (DWA) containing 15g agar per litre of distilled water was used as a minimal medium to induce sporulation.

Solid media used in fungicide experiments consisted of PDA amended with appropriate rates of fungicide solution.

Liquid media used in fungicide experiments were of the same composition as PDA, but without agar.

All media, before amendment with heat-sensitive chemicals, were sterilised at 121°C for 15 min.

2.1.4 Isolations of *Pseudocercosporella*

2.1.4.1 Mycelial isolates

Mycelial isolates were obtained directly from infected cereal stems. Stems were washed in tap water, rinsed in sterile distilled water, soaked in 1-2% sodium hypochlorite for 2 min and then washed twice in sterile, distilled water. Stems were dried between clean (unsterilised) paper towels. Sections were sliced from lesions or

mycelium taken from inner stem lumens. These were plated onto PDA amended with streptomycin and incubated at 18°C for 2-3 days until colonies had grown. Hyphal plugs were removed and placed onto unamended PDA, to prevent selection for antibiotic sensitivities.

2.1.4.2 Conidial isolates

Conidial isolates each originated from a single conidium. Conidial suspensions were streaked over DWA plates and individual conidia were removed with a fine scalpel while viewed using an inverted compound microscope.

2.1.5 Culture methods

2.1.5.1 Mycelial cultures

Agar cultures were grown in a 15°C incubator. Liquid cultures were continuously shaken on an orbital shaker maintained in a culture growth room kept at 20-25°C. Stock cultures were stored as slope cultures on PDA in McCartney bottles, at 5°C in the dark.

2.1.5.2 Conidiation in culture

Conidia were obtained by placing either infected lesions or PDA plugs of hyphae onto petri dishes containing moist filter paper or water agar, respectively. These were maintained at 5°C under continuous near ultraviolet light (Philips black light tube, model TL 20W/08, peak output 350nm) for 10-14 days. To obtain prolific sporulation, sterile, distilled water was poured over the agar cultures following one to two weeks incubation. Cultures were then incubated for another week.

2.1.5.3 Fungitoxicity tests

Subcultures of hyphal plugs from margins of actively-growing colonies on PDA were transferred to agar, or liquid culture, amended with an appropriate rate of fungicide. There were at least two replications of every test. Control tests were made on both unamended PDA and PDA amended with ethanol, at the rate used to dissolve fungicides in experimental media. Plates were incubated at 15°C.

A stock solution of benomyl (1000ug a.i. ml^{-1}) was made by dissolving 0.5g Benlate (50% benomyl) in 5ml ethanol and adding to 245ml sterile, distilled water.

Stock solutions of prochloraz (1000 ug a.i. ml^{-1}) were freshly made before use. They consisted of 0.5ml of 450g l^{-1} prochloraz (45EC Sportak) added to 224.5 ml sterile, distilled water. The solution was mixed using a magnetic stirrer hot plate (Chiltern Scientific, MM31) maintained at a low heat. A stock solution of DPX H6573(2000 ug a.i. ml^{-1}) was made by adding 1g DPX H6573 (containing 0.2 ug a.i.) to 100ml sterile, distilled water and mixing with a magnetic stirrer hotplate kept at a very low heat.

Appropriate quantities of stock solution were added to sterilised agar before pouring into petri dishes. Hyphal plugs (5mm) were removed from the margins of colonies actively growing on PDA and placed on experimental plates. Each treatment plate was duplicated except for the control plates of which sometimes only one was used. Plates were incubated at 15°C and growth was measured and recorded as the mean of two perpendicular colony diameters.

Concentrations used in the benomyl tests were:-

1 Control (unamended PDA)
2 0.02 ug a.i. ml^{-1} (100.0ml PDA + 0.01ml stock solution) = 0.02BEN PDA
3 0.20 ug a.i. ml^{-1} (100.0ml PDA + 0.02ml stock solution) = 0.2BEN PDA
4 2.00 ug a.i. ml^{-1} (99.8ml PDA + 0.20ml stock solution) = 2BEN PDA
5 20.0 ug a.i. ml^{-1} (98.0ml PDA + 2.00ml stock solution) = 20BEN PDA
6 200.0 ug a.i. ml^{-1} (80.0ml PDA +20.00ml stock solution) = 200BEN PDA

Concentrations used in the prochloraz tests were:-

1. Control (unamended PDA)
2. 0.1 ug a.i. ml^{-1} (100.00ml PDA + 0.01ml stock solution) = 0.1PRO PDA
2. 0.2 ug a.i. ml^{-1} (100.00ml PDA + 0.02ml stock solution) = 0.2PRO PDA
3. 0.5 ug a.i. ml^{-1} (99.95ml PDA + 0.05ml stock solution) = 0.5PRO PDA
4. 1.0 ug a.i. ml^{-1} (99.90ml PDA + 0.10ml stock solution) = 1PRO PDA
3. 2.0 ug a.i. ml^{-1} (99.80ml PDA + 0.20ml stock solution) = 2PRO PDA
6. 5.0 ug a.i. ml^{-1} (99.50ml PDA + 0.50ml stock solution) = 5PRO PDA

7. 10.0 ug a.i. ml^{-1} (99.00ml PDA + 1.00ml stock solution) = 10PRO PDA
4. 20.0 ug a.i. ml^{-1} (98.00ml PDA + 2.00ml stock solution) = 20PRO PDA
5. 200.0ug a.i. ml^{-1} (80.00ml PDA + 20.0ml stock solution) = 200PRO PDA

Concentrations used in the DPX H6573 tests were:-

1. Control (unamended PDA)
2. 0.2 ug a.i. ml^{-1} (500.0ml PDA + 0.05ml stock solution) = 0.2DPX PDA
3. 2.0 ug a.i. ml^{-1} (499.5ml PDA + 0.50ml stock solution) = 2DPX PDA
4. 20 ug a.i. ml^{-1} (494.5ml PDA + 5.50ml stock solution) = 20DPX PDA
5. 200 ug a.i. ml^{-1} (445.0ml PDA + 55.0ml stock solution) = 200DPX PDA

Liquid culture benomyl tests consisted of inoculated flasks of liquid media randomly arranged on an orbital shaker and continuously agitated. The shaker was located in a growth room kept at 25°C with a 16h day/ 8h night light regime (Osram 58W cool white 33 lights). After an appropriate time period, the flasks were removed and the mycelium filtered from the broth, using a Millipore Sterifil Aseptic System with Type AW prefilter (47mm diameter). The mycelium was dried in an oven kept at 75°C for 12h. Samples were cooled to room temperature in a vacuum desiccator and then weighed.

2.1.5.4 Responses of isolates to MDPC

A stock solution of 2000 ug a.i ml^{-1} MDPC was made by adding 2g MDPC (containing 0.5g a.i.) to 250 ml sterile, distilled water. This was added in appropriate quantities to flasks of PDA before pouring into petri dishes.

Treatments

1. Control (PDA)
2. 2.0 ug a.i. ml^{-1} (999ml PDA + 1ml stock solution)
3. 20.0 ug a.i. ml^{-1} (495ml PDA + 5ml stock solution)
4. 200.0 ug a.i. ml^{-1} (450ml PDA + 50ml stock solution)

Hyphal plugs of isolates were removed with a cork borer from the margins of colonies actively-growing on PDA. One plug of each isolate

was placed on a petri dish of each treatment, which was duplicated. Treatment plates were kept in a 15°C incubator for 14 days and growth was noted for each isolate as present or absent.

2.1.5.5 Effect of acenaphthene on *Pseudocercosporella*

The barrier technique used by Curtis *et al.*, (1956), whereby crystals of acenaphthene are directly sprinkled around isolates of *Penicillium chrysogenum* Thom, directly on culture media, was applied to isolates of *Pseudocercosporella*. Isolates were subcultured from stock cultures onto PDA and acenaphthene was sprinkled around the isolates and left for 20 days.

2.1.6 Phenotype classification

Isolates were phenotypically classified using the description of Scott *et al.*, (1975). Conidial measurements were made using an ocular micrometer on a compound microscope.

Feulgen staining

Conidia were air-dried onto glass microscope slides and fixed with 3:1 absolute ethyl alcohol and glacial acetic acid. Slides were immersed in 1N HCl at room temperature for 5 min and then 60°C for 13 min. Slides were washed in distilled water. Slides were soaked in glacial acetic acid/ 65% lactic acid and then counterstained in 1% lactic acetic orcein. Preparations were made permanent by soaking in 45% acetic acid and then put through an alcohol series; 40% ethanol, 80% ethanol and two rinses in absolute alcohol. The spores were immersed in a drop of euparol and a coverslip was gently lowered on to each slide. Slides were kept on a hotplate until the fixative had dried.

2.1.7 Electrophoresis

2.1.7.1 Preparation of culture samples

Mycelial colonies were scraped from the surface of PDA plates and mechanically homogenised in distilled water with an Ultra-turrax (Janke and Kunkel, Ika-Werk) blender. The homogenate was centrifuged at 1200g for 15 min and the supernatant removed with a syringe and freeze-dried to reduce the volume. The preparation was thawed immediately before use and 3-4 drops of water were added to reconstitute the soluble proteins.

2.1.7.2 Electrophoretic procedure

Gels were prepared by the method of Davis (1964), see Appendix 3. A large-pore stacking gel, to concentrate the samples, was prepared above a small-pore main gel. The gels were 1.5 mm thick and used in vertical electrophoretic apparatus containing a litre of tris glycine (pH 8.3). Sucrose and bromophenol blue were added to the culture preparations, the former to increase sample density for sharper staining and the latter so that movement of the samples through the gel could be followed. Samples (25 ul) were loaded into wells in the gel using a syringe. A 100V current was run through the gel for 2 h and this was increased to 150V for a third hour. The cathode was at the top and the anode at the bottom of the apparatus and so the sample anions moved downwards.

2.1.7.3 Esterase detection and gel fixation

After electrophoresis, the gels were soaked in a mixture of 5mg α-napthyl acetate (dissolved in 0.5ml ethanol) and 12mg fast-garnet (dissolved in 0.1M phosphate buffer, pH 7.2). α-napthyl acetate is used by the esterase as a substrate and the fast garnet is a coupling agent, resulting in staining of bands where esterase isozyme components occur. Gels were left for at least half an hour in the dark at 25°C while staining took place. The solution was then removed and the gels fixed in 7% acetic acid (pH 1) to denature the enzyme and then rinsed in distilled water.

2.2 *IN VIVO*

2.2.1 Plants

Seeds were obtained from the Cereals Section (Dr D. Wright), Gene Bank (Mr R. Cross) and Haploid Barley Programme (Mr R. Pickering) of Crop Research Division, DSIR, Lincoln. The Zadoks decimal code for growth stages (G.S.) of cereals (Zadoks *et al.*, 1974) is used to describe plants throughout the study.

2.2.2 Land/soil

Field trial sites were situated at DSIR, Lincoln, Canterbury and DSIR, Gore, Southland. The soil type at Lincoln was Wakanui silt loam and that at Gore, Waimumu silt loam.

Soil used in growth room trials was a sterilised mix of 3 parts pine bark and one part washed river sand.

2.2.3 Meteorological data

Rainfall and temperature data pertaining to the Lincoln trials were obtained from the Lincoln weather station H32641. Data pertaining to the Gore trials were obtained from the Gore weather station I68192.

2.2.4 Equipment and chemicals

2.2.4.1 Field trials

Wintersteiger seedmatic drill, General farm machinery, Fertiliser spreader, Hand-held CO_2 sprayer, Thresher (DSIR), Seedcounter, Mettler PE 3600 Balance, Secateurs. Nitrophoska (12 parts N:10P:10K), (BASF, NZ Ltd) Hoegrass (36EC) - Hoechst AG, W. Germany, Glean - Du Pont N.Z. Ltd

2.2.4.2 Growth room

Temperature 10°C
Lights: 12 h light regime - Sylvania fluorescence growth lamps (FR96T12/CW/VHO/235), irradiance = 450 uE m^{-2}

Relative humidity: 60%
Trickle irrigation system controlled by a Gardena 2010 computer
Thermohydrograph (Casella, London)
Pots (14cm diameter, 15 cm depth) and punnets measuring 20cm by 15cm, with depth 6.5cm were used. If not already present, holes were placed through the bases for drainage.

2.2.4.3 Glasshouse

Temperatures : 10°C at night, 18-25°C at day
Relative humidity: uncontrolled, approximately 75%

2.2.5 Sowing

2.2.5.1 Field

Seed was sown in the field using a Wintersteiger seedmatic seed drill. The six coulters and hence row widths were 0.17m apart. The plot width was 0.85m and as the tractor width was 1.5m, pathways between the plots were 0.65m. Plots were 1m in length and at Lincoln, 0.6m was left between each plot along a tractor run. Trials were rotary-hoed along the pathways perpendicular to the tractor runs and as the rotary hoe was 0.7m wide, the plot lengths were reduced to 0.9m.
Rotary-hoeing is undertaken to remove weeds from between the plots and to straighten the edges of the plots. At Gore, the land is too wet to rotary-hoe and pathways are mowed instead. As the mower is 1m in width, a wider pathway had to be left between the plots. Plot lengths were 1m, as the drill was tripped every 2m.

The barley used for the yield trial was broadcast at a rate of approximately 150kg ha^{-1}.

2.2.5.2 Growth room

Seeds were sown in pots or trays in 3:1 bark/sand and left in a glasshouse at 25°C for 10-14 days and watered daily. This allowed germination and subsequent seedling growth to G.S. 12-13. If plants had reached this stage before inoculum was ready, they were maintained, although not for more than a few days, at 5°C, to inhibit further growth.

2.2.6 Inoculum

Isolates used for inoculation were selected from a collection obtained from Southland cereal crops.

2.2.6.1 Field inoculation

Preparation of field inoculum

Inoculum was in the form of colonised oatgrains, similar to that described by Sprague (1936). Oat grains (50g) were boiled in each of ten 250ml flasks. Flasks were autoclaved for one hour at 121°C, left for 24 hours (shaken intermittently) and again autoclaved. Each flask was inoculated with six mycelial plugs from actively-growing isolates. Oatgrains (400g) and sterile, distilled water (400ml) were autoclaved for one hour on two consecutive days in clear, plastic, autoclavable bags and after one week at room temperature (21-22°C) the colonised grains were added to these at the rate of one flask per bag. After incubation for 5-6 weeks at 18°C, grains were well colonised and ready for immediate use. When inoculum was not immediately required, it was dried to approximately 14% moisture content, by leaving spread on trays in a drying oven for a few days. The oven temperature thermostat was left off to maintain grain at ambient room temperature thus not affecting the *Pseudocercosporella*. A fan was left on to facilitate drying.

Inoculation

Plants were inoculated at G.S.12 to prevent toxic effects on germinating seeds and stunting of the plants. Grain was hand-sprinkled onto the field at the rate of $15\,g\,m^{-2}$. Measurement was made by converting this from weight to volume and applying grain from a graduated glass tube.

2.2.6.2 Growth room inoculation

Inoculum consisted of hyphal plugs taken from colonies actively-growing on PDA. One plug was placed on each seedling with hyphae touching the base of the stem. A short piece of split plastic

drinking straw (milkshake straw) was placed around each stem base to hold the inoculum in place. Soil level was raised to cover 3/4 of each straw.

2.2.7 Scoring

2.2.7.1 Growth room

Disease was scored on a scale describing the number of leaf sheaths infected.

0 = no infection
1 = first leaf sheath infected
2 = second leaf sheath infected
3 = third leaf sheath infected
4 = fourth leaf sheath infected
5 = complete stem infection - implies lodging would occur if stem not supported by plastic drinking straw

A half value designates stromatal hyphae having reached the next inner leaf sheath, but not having yet infected it. Plants were scored after 6-7 weeks.

2.2.7.2 Field

Disease was scored on a scale similar to that used in the growth room, however more leaves were present in the field so the range of scores was wider.

0 = no infection 9 = complete infection, with lodging.

Half values were again incorporated. Plants were scored at G.S. 65-70.

2.2.8 Statistics

A Genstat computer package was used for determining analyses of variance and regressions.

EXPERIMENTAL WORK PART I - Distribution and description of the *Pseudocercosporella* population

3.0 PRESENCE AND DISTRIBUTION OF *PSEUDOCERCOSPORELLA* IN CEREAL-GROWING REGIONS OF THE SOUTH ISLAND OF NEW ZEALAND

The presence and distribution of *Pseudocercosporella* in Southland, N.Z. was determined by conducting surveys of cereal crops during the 1984/85 and 1985/86 seasons.

The results of a preliminary investigation made during 1983/1984 are listed in Table A1.1 and is used for comparison.

3.1 SAMPLING METHOD

Wheat, barley, rye and triticale crops in Southland were sampled when milky ripe (G.S. 71-79). The first samplings were made during January, 1985 and the second in December, 1985 and January, 1986. During the 1985/86 survey, samples were also collected in Central Otago and Canterbury. Sampled sites were randomly chosen, however sites known to have been either sprayed with prochloraz or to have a long history of benomyl usage, were included in the surveys ensuring a wide range of samples available for later fungicide studies. Wherever possible, complete paddock management histories were obtained and the same sites sampled each season. Ten samples, of at least ten tillers each, were removed from each field. Well-spaced samples were taken at random with both lodged and standing tillers being removed from partially lodged crops. Tillers were up-rooted, and the stem bases stored in paper bags. Percentage tiller infection was scored for each sample.

3.2 RESULTS AND DISCUSSION

Wheat and barley crops predominated over the three seasons (Table 3.1) with numbers sampled being about equal in the first two, however the number of barley crops increased in the third, reflecting the increased number of hectares sown in barley that season. Details are included as Appendix 1 (Tables A1.1-A1.3).

Sites sampled included a range of cropping histories (Table 3.2). As prochloraz was only introduced in the 1984/85 season, few sites would have been sprayed and none were sampled until 1985/86 (Table 3.3). A larger number of sites with a benomyl history were also sampled in that season, reflecting greater chemical usage. The incidence of eyespot varied between these two surveys (Table 3.4), as a result of different weather patterns.

In the 1984/85 survey, following a rainy summer, the majority of sites had more than 50% of sampled tillers infected. Eyespot lesions were frequently found high on the stems and lodging was more prevalent, indicating conditions were extremely conducive to disease development. In the following season, when conditions were very dry, infection rates tended to be less than 50%.

Lodging was more frequent in the 1984/85 season, more particularly in wheat crops and most probably resulted from combined effects of eyespot and rain.

Lower tiller infection rates were found in Otago and Canterbury, areas less prone to eyespot, with warmer and drier conditions. Frequently, once a field is found to contain eyespot, it is subsequently sown with a break crop or put back into pasture.

Table 3.1

Types and numbers of cereal crops sampled

	1983/84 Southland	1984/85 Southland	1985/86 Southland	Central Otago	S. Canterbury	N. Canterbury	Total
Wheat	34	15	29	2	4	7	91
Barley	30	16	53	0	5	10	114
Triticale	0	3	3	0	0	0	6
Oats	2	1	2	0	0	0	5
Ryecorn	1	0	0	4	0	1	6
Ryegrass	0	0	1	0	0	0	1
Total	67	35	88	6	9	18	223

Table 3.2

Southland cropping histories for 1984/85 and 1985/86 surveys

Seasons	Number of sites sampled with cereal history of:-						
	1st yr (*)	2nd yr (*)	3rd yr (*)	4th yr (*)	5th yr (*)	>5th yr (*)	Unknown
1984/85	4	5	4	3	3	1	0
1985/86	13 (4)	13 (5)	4 (2)	7 (1)	7	15	0
Total	17 (4)	18 (5)	8 (2)	10 (1)	10	16	0

* indicates that prior to this a break crop had been sown, following an earlier sequence of cereals.

Table 3.3

Spraying histories of sampled sites

Year	Number of sites with a history of :- benomyl	prochloraz	benomyl + prochloraz	no sprays	unknown
1984/85	13	-	-	10	12
1985/86	32	5	8	9	31
Total	45	5	8	19	43

Table 3.4

Incidence of eyespot at sampled sites

Season	Number of sampled sites with percentage tiller infection rates of:- >50%	<50%	no infection	unknown	total
1984/85 Southland	20 (57.10%)	2 (5.71%)	1 (2.85%)	12 (34.28%)	35
1985/86 Southland	16 (0.18%)	59 (67.05%)	0 (0.00%)	13 (14.77%)	88
Central Otago	0 (0.00%)	3 (50.00%)	0 (0.00%)	3 (50.00%)	6
S. Canterbury	1 (11.11%)	4 (44.44%)	0 (0.00%)	4 (44.44%)	9
N. Canterbury	0 (0.00%)	15 (83.33%)	0 (00.0%)	3 (16.66%)	18
Total	37	83	1	35	156

4.0 MORPHOLOGICAL STUDY

4.1 METHOD

Isolations were made from as many infected tillers as possible, however, not more than one isolate was taken from each tiller. Stock cultures were maintained as slant cultures on PDA at 5°C in the dark.

Isolate morphology

Where possible, isolates were characterised as SF or FE on the basis of colony morphology on PDA. General characteristics were also described as isolate variability was noted within these groups. Conidial length was determined for a sample of 24 isolates by measuring 30 conidia per isolate. Morphological variability among isolates within the population was also examined.

Feulgen staining

Conidia from isolate 86/2SC/14 (FE) were stained with Feulgen and observed under an oil-immersion lens (1 000x).

4.2 RESULTS

In the preliminary 1983/84 survey, some 95% of isolates collected on both wheat and barley crops were of the FE growth type (Table 4.1). The five isolates collected from wheat during the 1984/85 season were FE as were 50% of the 14 isolates from barley. This suggests an increase in number of SF isolates on barley however the sample size of that survey was small. In 1985/86, with a greater number of isolates from wheat, 73% were FE and of 6 isolates collected from barley, 5 (83%) were FE. Descriptions of isolates are listed in Appendix 2.

Table 4.1 Summary of known isolate types collected from wheat, barley and triticale crops in annual surveys.

	WHEAT			BARLEY			TRITICALE			TOTAL
	FE	SF	Total	FE	SF	Total	FE	SF	Total	
1983/84*	21 (95)	1 (5%)	22	32 (94%)	2 (6%)	34	-	-	-	56
1984/85	5 (100%)	0 (0%)	5	7 (50%)	7 (50%)	14	-	-	-	19
1985/86	36 (73%)	13 (27)	49	5 (83%)	1 (17%)	6	2 (100)	0	2	57

* Data collected prior to current study (see Appendices 1 and 2)

Descriptions of morphologies were based only upon the appearance of colonies growing on PDA. A variety of colony morphologies were obtained (Fig. 4.1), with FE isolates being the easier type to classify. SF isolates included not only feathery isolates which frequently grew at a comparatively fast rate, as is exemplified by isolate G63998 (Fig. 4.2) but also slow-growing isolates with fairly even edges and usually convoluted colonies, such as isolates 85/7/1, K15 1 (Fig. 4.1) and G64344 (Fig. 4.2).

Fig. 4.1 A range of isolates collected in Southland during 1984/85, showing the variation in colony morphology on PDA.

From left to right and top to bottom: Isolates K2 2, K1, 85/7/1, K26 1, 85/3/1, K15 1, K2 3, K21 1 and 85/4/1.

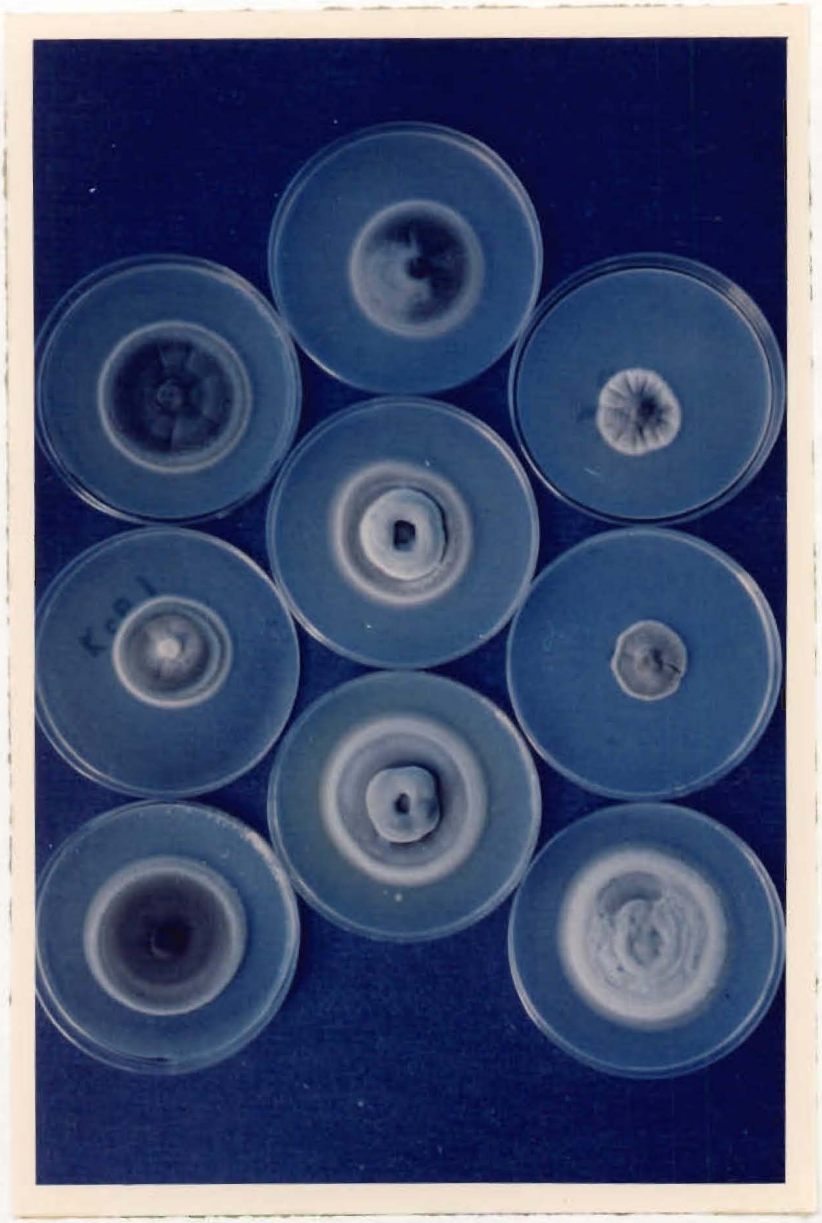
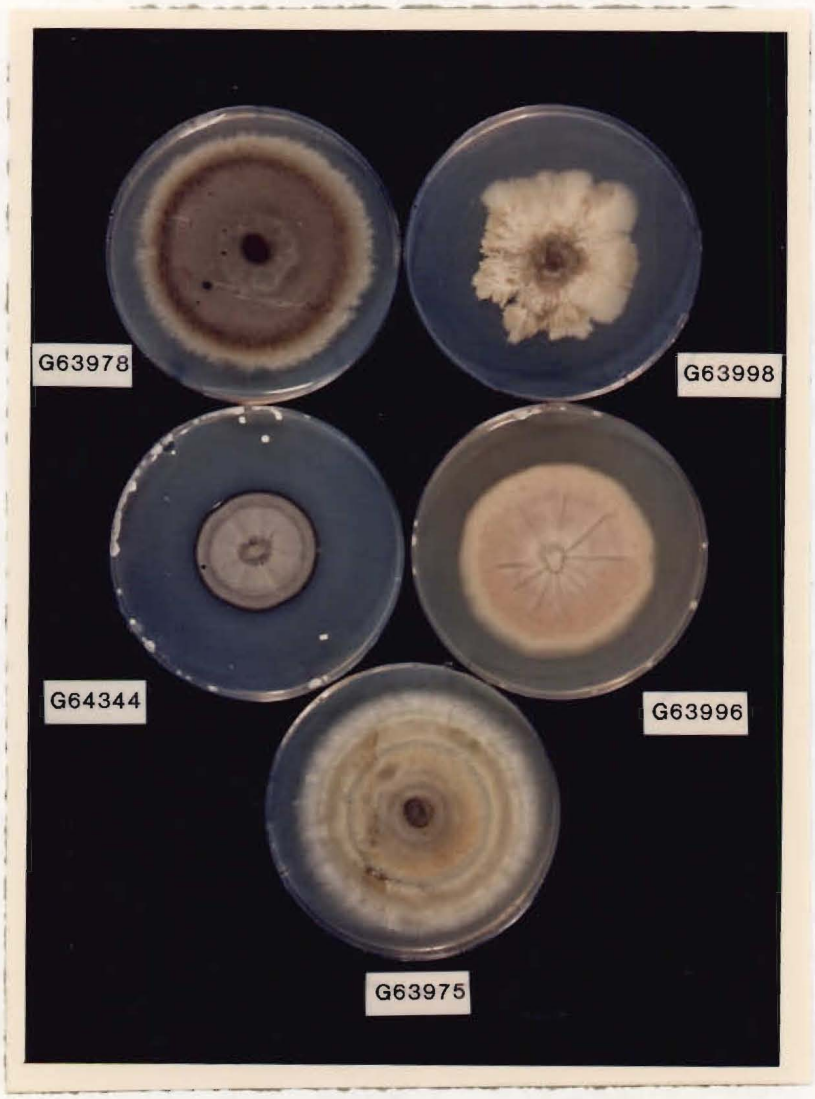


Fig. 4.2 Colony morphologies of the species described by Nirenberg (1981).

G63978 - *P. herpotrichoides* var. *herpotrichoides* (conidia straight)
G63998 - *P. herpotrichoides* var. *acuformis*
G64344 - *P. aestiva*
G63996 - *P. anguioides*
G63975 - *P. herpotrichoides* var. *herpotrichoides* (conidia curved)



In an attempt to be more precise with the classification of isolates, measurements of conidia were taken (Table 4.2, Fig. 4.3) and these ranged from 24-102u. All isolates had conidia falling within the ranges decribed by *P. herpotrichoides* var. *herpotrichoides* and *P. herpotrichoides* var. *acuformis*. Straight and curved conidia were found within both individual FE and SF N.Z. isolates. Conidia typical of *Pseudocercosporella* are shown in Fig. 4.4.

Table 4.2 Descriptions of conidia from a sample of isolates collected in the 1985/1986 season

CONIDIA

Isolate	Range (u)	Mean (u)	Standard Deviation (u)	Type
86/5/6	38.36-68.50	53.16	8.22	SF
86/33/1	54.80-101.38	74.53	11.04	SF curved, 0.5 width of other conidia
86/36/1	26.03-68.50	42.20	11.35	FE straight
86/36/2	24.66-73.98	57.45	12.22	FE straight, 1 curved
86/36/3	27.40-71.24	51.79	11.90	FE straight and curved
86/36/4	38.36-73.98	57.95	9.27	SF straight
86/36/5	49.32-82.20	62.20	8.98	SF
86/36/6	27.40-68.50	50.42	10.76	SF straight
86/36/7	46.58-93.16	70.33	9.69	SF straight
86/36/9	27.40-84.94	52.06	14.97	SF
86/36/12	32.88-95.90	55.90	10.12	FE straight
86/57/6	30.14-93.16	63.39	17.87	SF straight and curved
86/57/14	32.88-95.90	70.10	15.65	-
86/57/16	41.10-87.68	56.95	9.92	SF
86/78/12	35.62-90.42	61.92	12.10	-
86/81/2	32.88-65.76	51.79	7.73	FE
86/86/3	35.62-84.94	53.12	12.54	FE straight
86/86/31	52.06-82.20	68.77	7.42	-
86/2SC/2	32.88-71.24	54.85	11.66	FE
86/2SC/3	30.14-71.24	49.18	10.36	FE budding conidia
86/2SC/4	27.40-68.50	52.88	9.28	FE
86/2SC/11	52.06-68.50	60.10	4.93	SF
86/2SC/12	41.10-54.80	48.54	3.52	FE
86/2SC/14	38.36-60.28	51.32	7.15	FE

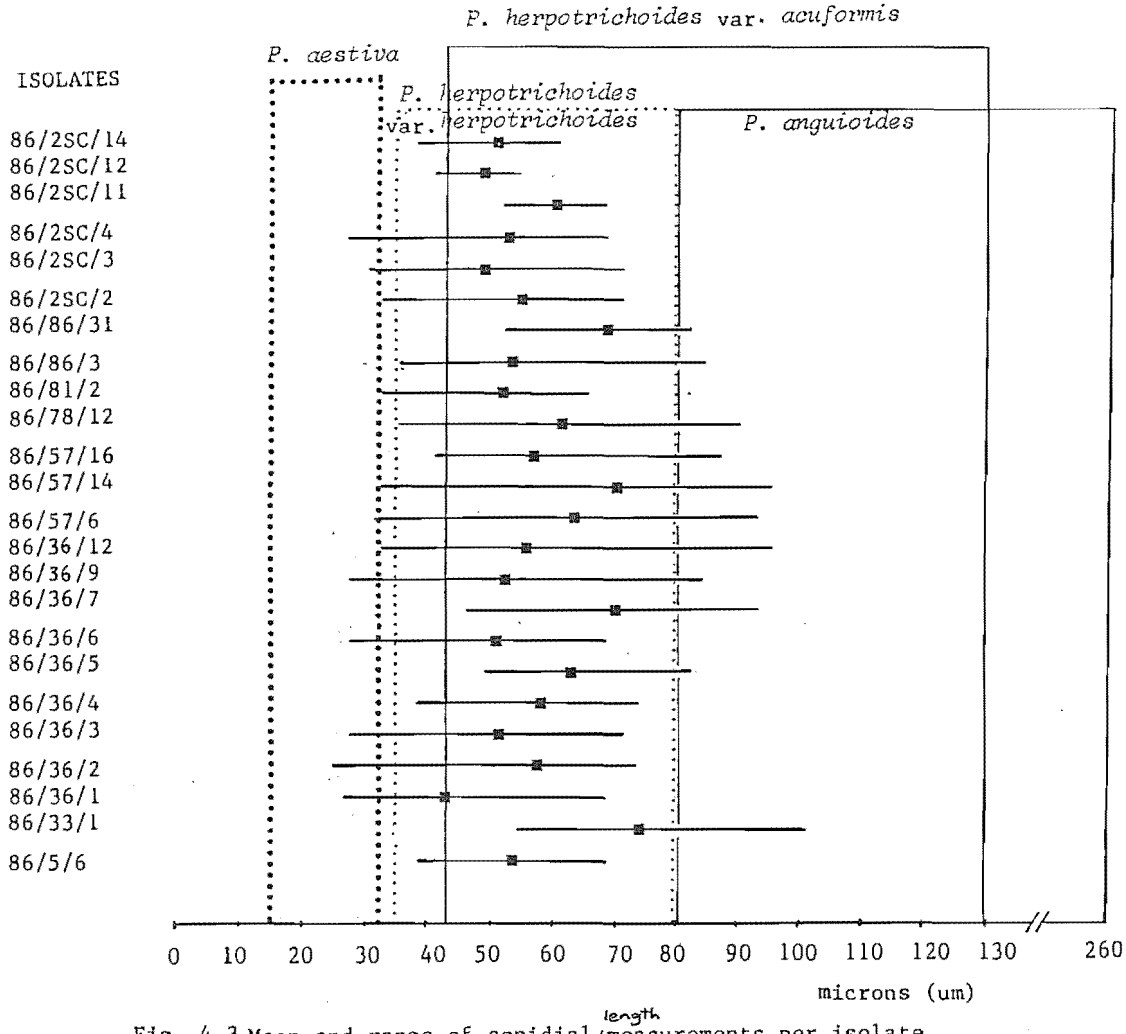


Fig. 4.3 Mean and range of conidial length measurements per isolate compared with ranges described by Nirenberg (1981)

Feulgen staining of conidia showed each cell within a conidium to contain one nucleus (Fig. 4.5).

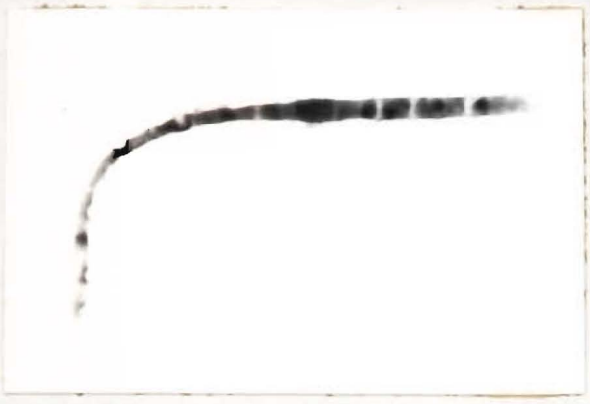
A SF isolate was found to inhibit the growth of a FE isolate (Fig. 4.6).

Fig. 4.4 Conidia of a N.Z. FE isolate (400X)

Fig. 4.5 (a) and (b)

Conidia of isolate 86/2SC/14 (FE) - Feulgen-stained and viewed under oil-immersion lens (1000X), to show a single nucleus per cell.

A



B

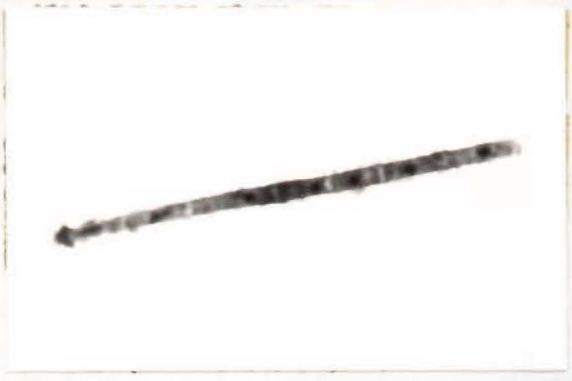


Fig. 4.6 Inhibition of a FE isolate by a SF isolate on PDA.

4.3 DISCUSSION

Over the three survey years, FE isolates remained the predominant type and numbers of SF isolates were not significantly higher on barley than on wheat. The increase in SF isolates may have coincided with the increased amount of barley grown and may reflect the UK trends (King and Griffin, 1985; Scott and Hollins, 1985) which however, are much more dramatic.

When stems were only mildly infected it was more difficult to isolate the pathogen, as fungi such as *Fusarium* spp grew faster than *Pseudocercosporella*.

When studying individual isolate variation, mass-mycelial isolates provide difficulties. Growth type, for example, may not be as distinct as SF or FE. A diploid "hybrid" was suggested (Davies and Jones, 1970) to have formed after hyphal fusion between two dissimilar isolates; a slow-growing black colony and a fast-growing white colony. This "hybrid" was grey and not able to sporulate.

Nuclei within a conidium are likely to be identical as they are asexually produced. If this is not so, a single hyphal tip from a conidium would be the preferred origin of an isolate to ensure an isolate originates from only one nucleus. The removal of these was found to be a too time-consuming task for the present purpose.

Colony appearances (Fig. 4.2) and hyphal growth rates (Table 1.1) of the two isolates, G64344 (*P. aestiva*) and G63998 (*P. herpotrichoides* var. *acuformis*), are similar to those of SF morphology. Ranges of conidial sizes of *P. herpotrichoides* var. *herpotrichoides* and *P. anguioides* do not overlap and as conidia from individual isolates, 86/36/6, 86/36/9 and 86/57/6 fell in both groups, the separation is questionable. Similarly, measurements of conidia from isolates 86/36/12 and 86/86/3, fell within the ranges of both *P. herpotrichoides* var. *herpotrichoides* and *P. anguioides*. The morphology and growth rates of these two species have also been described by Nirenberg as being similar. Nirenberg (1985) stated that *P. anguioides* and *P. aestiva* were less commonly found. It is possible that these are deviant isolates from the populations of the other two species.

It is possible that there are species differences between N.Z. isolates but their descriptions could be masked by natural variation existing between N.Z. and European populations.

Of the 24 isolates studied, spore length did not readily identify the species, hence it is not a useful character and alternatives are required.

The finding of a SF isolate capable of inhibiting the growth of a FE isolate was of interest. Assuming this inhibition also occurs *in vivo*, it is likely to confer a competitive advantage on the SF isolates.

With genetic crossings possible now that a sexual stage has been found, further insight may be gained into the taxonomy of the fungus.

5.0 BIOCHEMICAL DIFFERENTIATION OF *PSEUDOCERCOSPORELLA* ISOLATES

5.1 INTRODUCTION

Electrophoretic studies of the products of gene expression have provided information on variation within numerous fungal genera, species and form species. Protein patterns of soluble proteins have been found useful for distinguishing species of *Pythium* (Clare, 1963) and *Septoria* (Durbin, 1966). Patterns obtained were similar for isolates of different ages and from different locations.

The use of polyacrylamide gels instead of starch gels has allowed greater resolution of bands (Chang *et al.*, 1962). Esterase isozymes produced more varied banding than did total proteins for *Phytophthora* species, and hence more precision for comparisons (Hall *et al.*, 1969). *P. cinnamomi* Rands and *P. palmivora* (Butler) Butler possess amphigynous antheridia whereas *P. cactorum* (Lebert & Cohn) Schroeter possesses paragynous antheridia. No common band was found in the protein patterns of the first two species, suggesting that none of the proteins detected had a close bearing on the development of amphigynous antheridia, or perhaps any morphological pattern. This further illustrated variability encountered within these species. Gill and Powell (1968) were unable to distinguish races of *P. fragariae* Hickman.

Esterases have also been used for distingushing *Fusarium* species. Meyer *et al.*, (1964) found culture media had an effect on esterase patterns of one form species of *F. oxysporum* Schl. ex Fr. and suggested the enzyme may be induced by the host and be involved in host pathogenicity. It was emphasised that a large number of isolates from geographically diverse locations should be studied to determine variation within each form species. Meyer and Renard (1969) were unable to separate form species of *F. oxysporum*.

Protein patterns were found sufficient to separate two of the three varieties of the take-all fungus, *Gaeumannomyces graminis*. Culture media and age of mycelium also affected the patterns. Isolates of *G. graminis* could not be distinguished using esterase or peroxidase isozyme patterns, as variability was too great (Abbott and Holland, 1975).

Gairola and Powell (1971) attempted to identify isolates of *Leucostoma* spp, in the absence of their two distinguishing perfect states, by comparing protein patterns with known reference samples. Each isolate produced a unique protein pattern and it was proposed that this variation reflected the natural variation existing amongst the fungi and the existence of intermediate or related forms. If the genes encoding production of these proteins are segregating in a sexually-reproducing ascomycetous population, such variation is perhaps not surprising. In addition to this, studies with cereal rust fungi (Burdon and Roelfs, 1985; Newton *et al.*, 1985) have compared the diversity of isozymes apparent in sexual and asexual populations. Greater variation was found in the genetic structure of sexual populations of *Puccinia graminis* Pers. f. sp. *tritici* Eriks & Henn. whereas asexual populations were more clonal. There is no known sexual stage of *P. striiformis* West and Australian populations of *P. graminis* f. sp. *tritici* and *P. recondita* Rob. ex Desm. f. sp. *tritici* Eriks & Henn. are maintained through asexual reproduction (Watson, 1981). No variation in isozymes was detected in each of the three species.

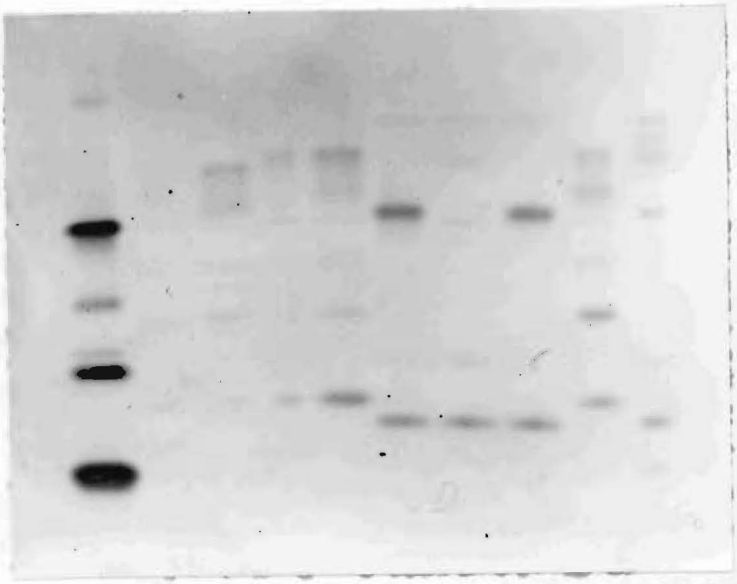
5.2 METHOD

A range of *Pseudocercosporella* isolates were tested for esterase isozymes using electrophoresis. (All N.Z. isolates were collected in Southland unless otherwise indicated). An isolate of *Mycosphaerella graminicola* Fuckel (Schroeter) was used for comparison.

5.3 RESULTS

Fig. 5.1 Esterase gel of isolates:

A. *Mycosphaerella graminicola*
B. 86/5/3 (FE)
C. 86/87/1 (FE)
D. GHRS8/D (FE)
E. 86/2SC/7 (FE)
F. 86/36/4 (SF)
G. 86/78/11 (SF)
H. GHRS8/E (SF)
I. FE mixture
J. SF mixture



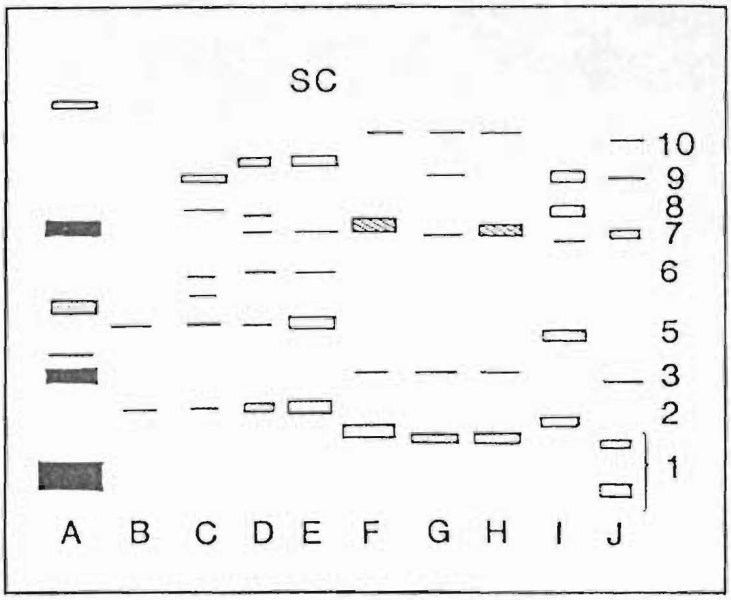
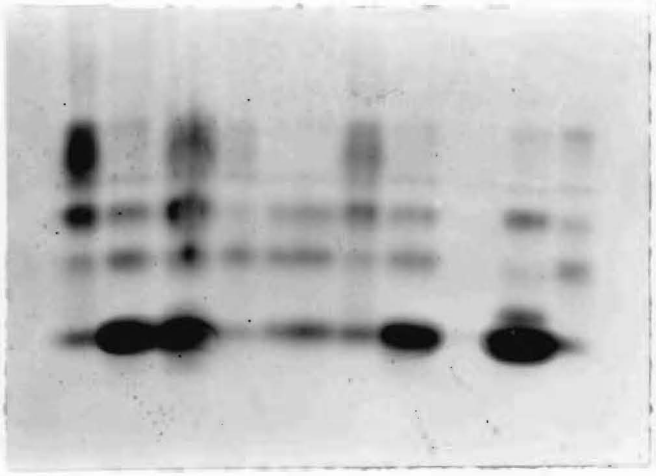

SC
10
9
8
7
6
5
3
2
1
A B C D E F G H I J

Fig. 5.2 Esterase gel of isolates:

A. 86/1SC/2 (FE)
B. 86/32/1 (FE)
C. 86/5/4 (FE)
D. GHRS2/8 (FE)
E. 86/5/1 (FE)
F. 86/1SC/2 (FE) *
G. 86/32/1 (FE) *
H. 86/32/3 (FE)
I. 86/86/7 (FE)
J. 86/86/6 (FE)

* denotes a duplicate

SC - South Canterbury isolate



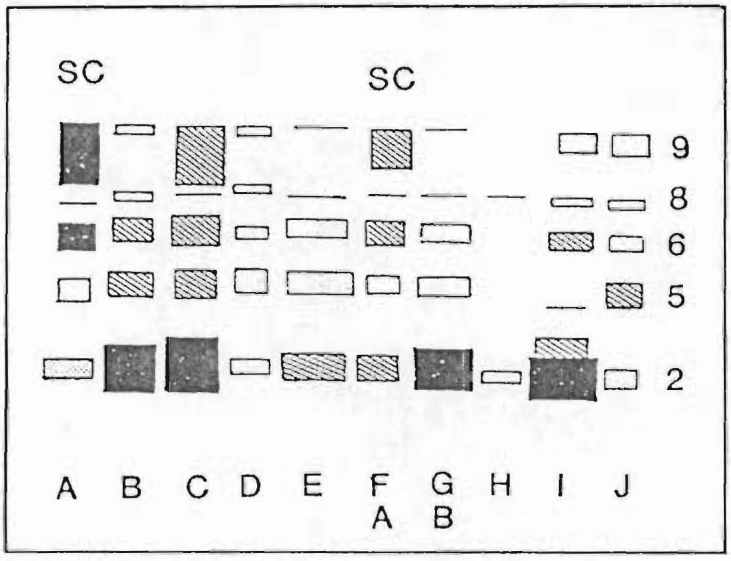
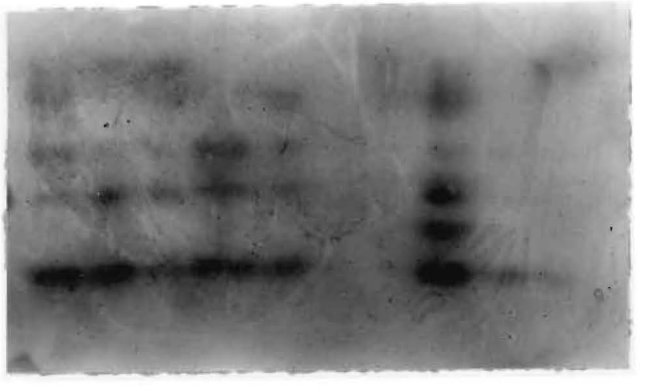

SC
SC
9
8
6
5
2
A
B
C
D
E
F
A
G
B
H
I
J

Fig. 5.3 Esterase gel of isolates:

A. 86/2SC/8 (FE)
B. 86/86/5 (FE)
C. 86/81/3 (FE)
D. 86/2SC/11(FE)
E. 86/36/3 (FE)
F. 86/2SC/5 (FE)
G. 86/NC17/1(FE)
H. 86/5/7 (FE)
I. 86/2SC/9 (FE)

SC - South Canterbury isolate

NC - North Canterbury isolate



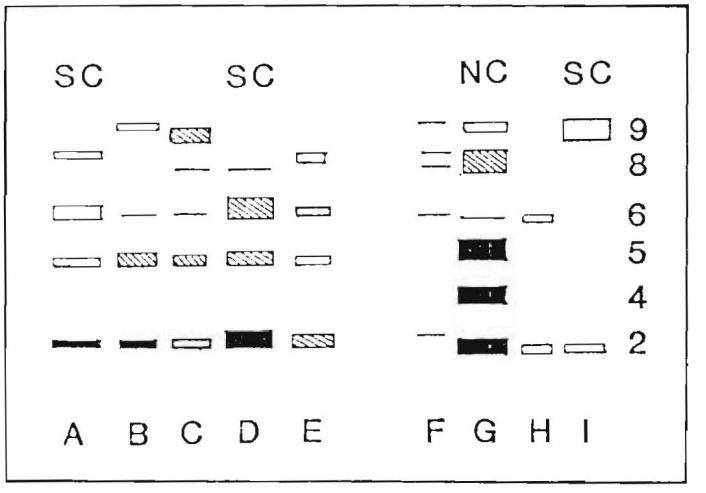
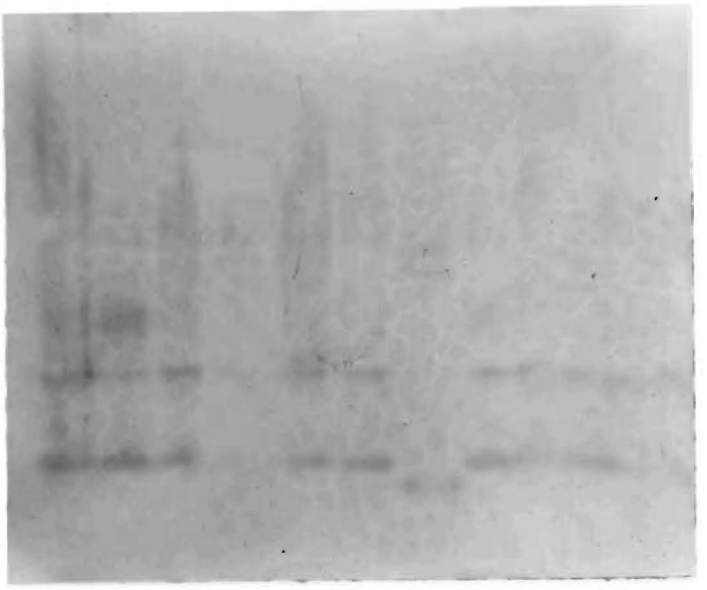

SC
SC
NC
SC
9
8
6
5
4
2
A
B
C
D
E
F
G
H
I

Fig. 5.4 Esterase gel of isolates:

A. 86/3/1 (FE)
B. 86/2SC/11 (FE)
C. 86/2SC/37 (FE)
D. 86/87/3 (-) ----FE bands
E. 86/86/18 (FE)
F. 86/86/100 (SF)
G. 86/2SC/1 (FE)
H. GHJA1/4/3 (FE)
I. 86/86/18 (FE) *

* denotes a duplicate

SC - South Canterbury isolate



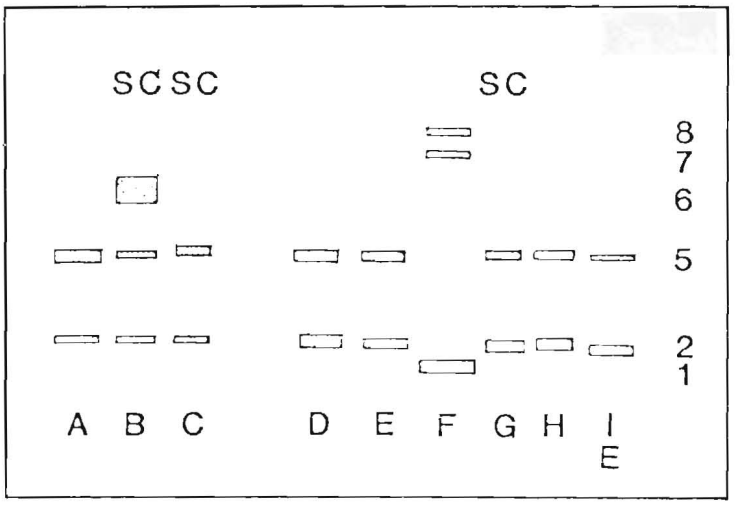
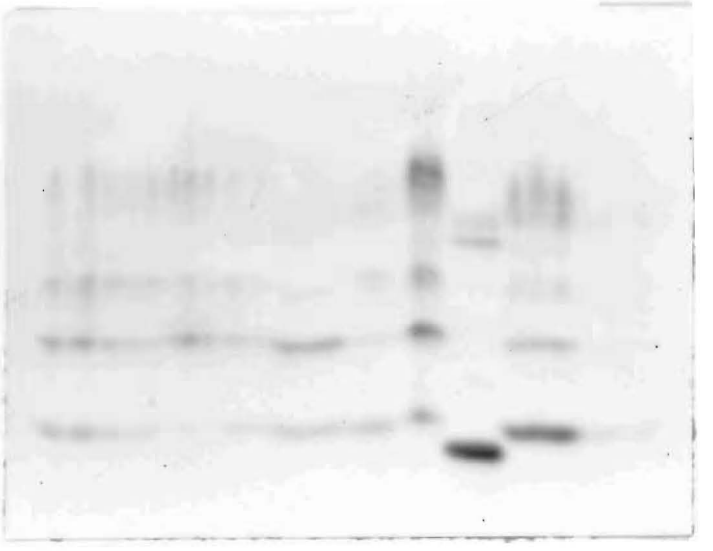

SC SC
SC
8
7
6
5
2
1
A B C D E F G H I
E

Fig. 5.5 Esterase gel of isolates

A. 86/86/17 (FE)
B. 86/86/31 (-) ----FE bands
C. 86/86/34 (FE)
D. 86/36/11 (FE)
E. 86/86/22 (FE)
F. 86/86/20 (FE)
G. 86/86/100 (SF)
H. 86/2SC/5 (FE)

SC - South Canterbury isolate



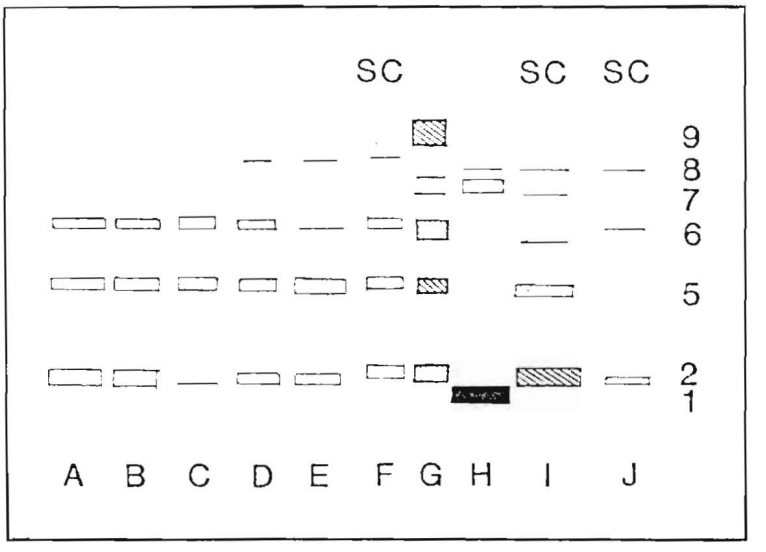
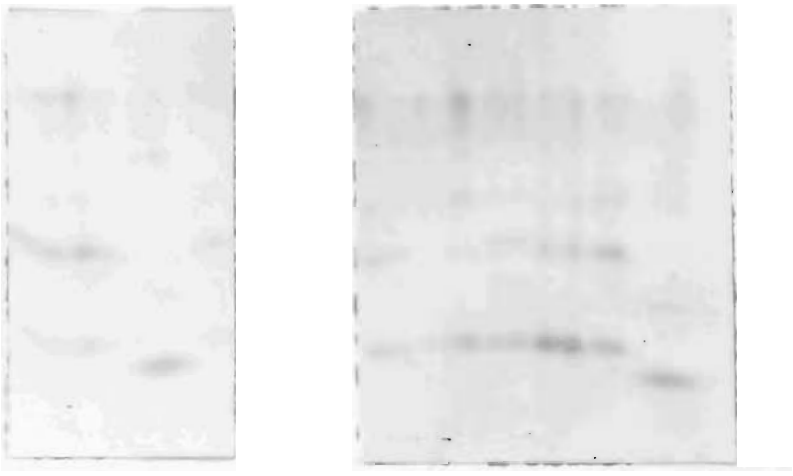

SC
SC
SC
9
8
7
6
5
2
1
A
B
C
D
E
F
G
H
I
J

Fig. 5.6 Esterase gel of isolates

A. 86/86/16 (FE)
B. 86/78/21 (FE) ----SF band
C. 86/33/2 (FE)
D. 86/33/2 (FE) *
E. 86/86/26 (FE)
F. 86/NC17/1 (FE)
G. 86/81/2 (FE)
H. 86/5/5 (FE)
I. 86/36/5 (SF)

* denotes a duplicate

NC - North Canterbury isolate



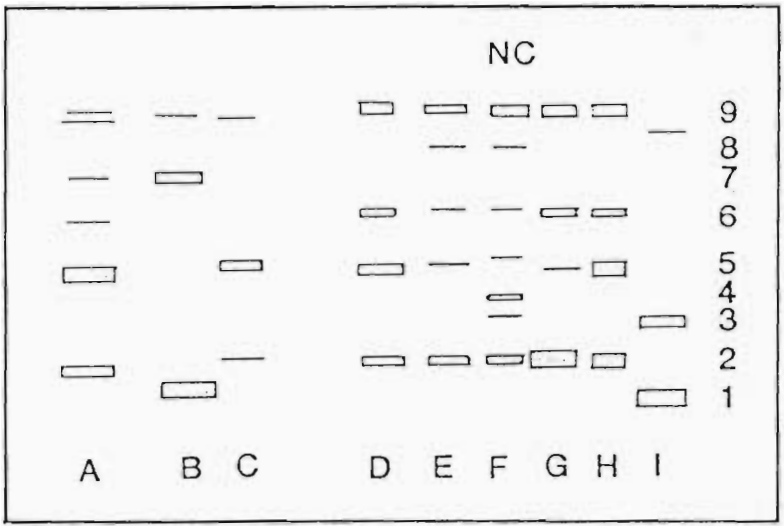
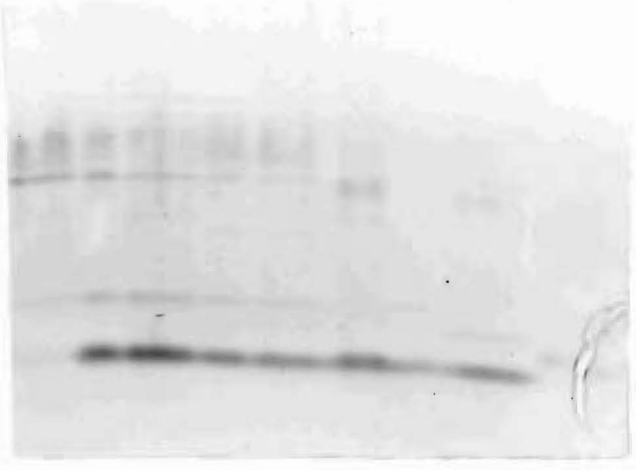

NC
9
8
7
6
5
4
3
2
1
A
B
C
D
E
F
G
H
I

Fig. 5.7 Esterase gel of isolates

A. 1/2 (feathery-fast)
B. 4/1 (SF)
C. 1/100 (FE, but culture mixed with 1/1 and 1/2 (SF))
D. 5/6 (SF)
E. 5/5 (SF)
F. 5/3 (SF)
G. 1/1 (feathery-fast)
H. 5/1 (SF)
I. 3/6 (FE)
J. 3/5 (FE)

E = English isolate
G = German isolate



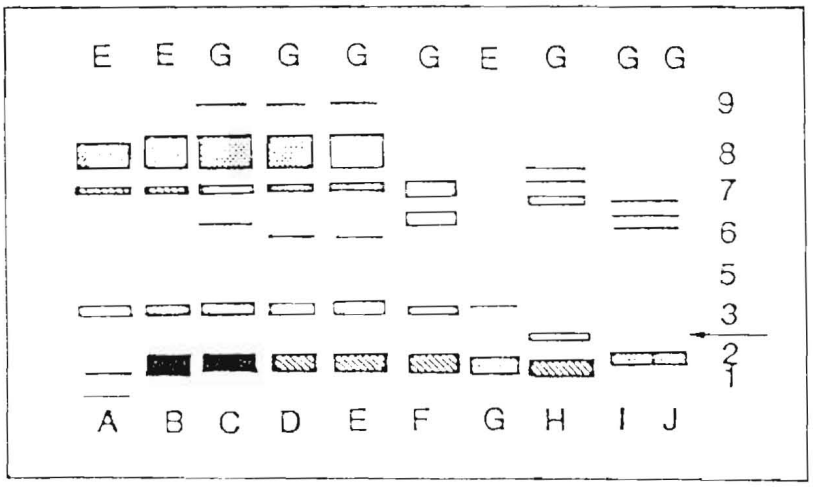

E E G G G G E G G G
9
8
7
6
5
3
2
1
A B C D E F G H I J

Fig. 5.8 Esterase gel of isolates

A. Australian isolate (from Mr J. Harris)

B. G63975 *P. herpotrichoides* var. *herpotrichoides* (curved conidia)

C. G63978 *P. herpotrichoides* var. *herpotrichoides* (straight conidia)

D. G63996 *P. anguioides*

E. G63998 *P. herpotrichoides* var. *acuformis*

F. G64344 *P. aestiva*

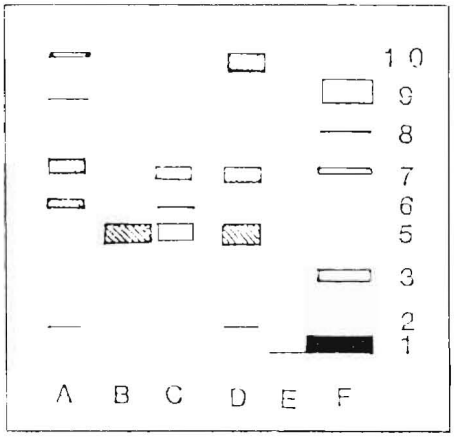
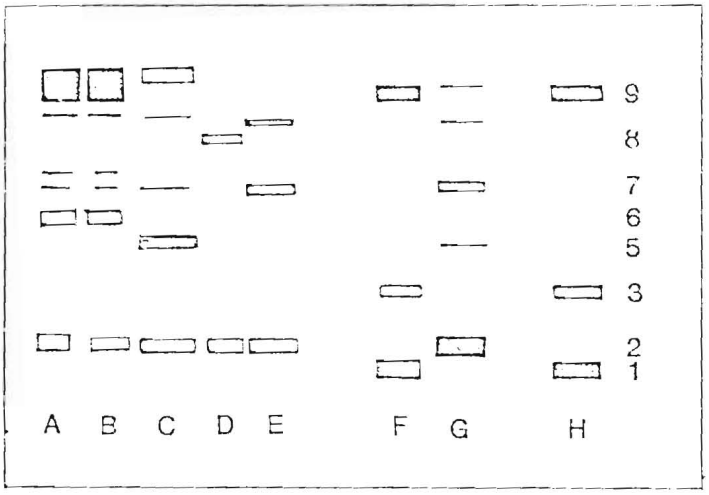

10
9
8
7
6
5
3
2
1
A
B
C
D
E
F

Fig. 5.9 Esterase gel of isolates

A. Single-ascospore isolate (from Dr. H. Wallwork)
B. Single-ascospore isolate*
C. Australian isolate (from Mr J. Harris)
D. GHRS2/1
E. G63978 *P. herpotrichoides* var. *herpotrichoides* (conidia straight)
F. G63998 *P. herpotrichoides* var. *acuformis*
G. G63978 *P. herpotrichoides* var. *herpotrichoides* (conidia straight)
H. G63998 *P. herpotrichoides* var. *acuformis*

*denotes a duplicate


9
8
7
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C
D
E
F
G
H

Photographs and diagrams of the gels are presented in Figs 5.1-5.9. Bands obtained were arbitrarily numbered 1-10 for reference. The number of FE *Pseudocercosporella* isolates tested reflects the predominance of this type within the N.Z. population, the European samples being predominantly SF. The isolate of *M. graminicola* produced a banding pattern distinctly different to those produced by *Pseudocercosporella* isolates. Age of either mycelium or frozen homogenate had no effect on banding patterns.

N.Z. FE isolates repeatedly produced bands 2 and 5 and frequently also 6 and 9. The isolate of *P. herpotrichoides* var. *herpotrichoides*, G63978 (FE), also produced bands 2, 5 and 6. N.Z. SF isolates produced bands 1 and 3 and frequently also band 7 and/or 9. *P. herpotrichoides* var. *acuformis*, G63998 (SF), produced bands 1, 3 and 9. Numerous minor bands were also produced. European isolates 1/1 and 1/2, were not similar to N.Z. isolates, having feathery colony edges yet fast growth rates. Both isolates produced 'SF' banding patterns, although isolate 1/1 produced a band between band positions 2 and 3 instead of a band at position 3. Isolate 5/5 (SF) produced band 6, expected of a FE isolate. Isolate 1/100 (FE) produced SF bands but the hyphae used in the gel was obtained from a plate of mixed isolates.

The one North Canterbury isolate (FE) produced a major band at position 4 which was not obtained for any other isolate. Isolate 86/78/21 which was classified visually as a FE isolate, produced a SF banding pattern and on re-examination, this was correct.

The single ascospore isolate produced bands 2 and 6, typical of FE isolates but instead of the expected band 5, produced a band between 5 and 6. The other Australian isolate produced bands 2, 5, 6, 8 and 9 as expected of a FE isolate. *P. anguioides* produced bands 2, 5 and 7, but *P. aestiva* produced a very strong band at position 1, a major band at position 3 and minor bands at positions 7, 8 and 9. These bands are typical of SF isolates.

5.4 DISCUSSION

The technique was successful in differentiating groups of isolates of *Pseudocercosporella*, with differences between morphological types and location of origin being detected. The fungus *M. graminicola* used for comparison showed major differences in banding.

Where bands are not present, it may be because insufficient isozyme was present for the stain to detect, rather than because the isozyme was absent. The technique differentiated SF from FE isolates and in one instance (isolate 86/78/21) more accurately described an isolate than an original visual examination. Apart from some variation between minor bands, the patterns obtained were consistent for each group. *P. herpotrichoides* var. *herpotrichoides* and *P. anguioides* produced bands expected of FE isolates and *P. herpotrichoides* var. *acuformis* and *P. aestiva* produced bands expected of SF isolates. Variation was obtained between isolates from different locations with the North Canterbury isolate and one of the German isolates each producing a band at a respectively unique position. The variation in morphology and banding patterns produced by the N.Z. and European isolates suggests that the bands relate more to colony morphology, such as the appearance of colony edge, than to growth rate. The convoluted isolates, with even colony edges and slow growth rate (such as *P. aestiva*), however, also produced typical SF banding patterns.

The Australian isolate, originating from a single ascospore, produced FE type bands, but with a unique band in place of one of the expected FE bands. If a large number of isolates from different populations were tested for esterases and other isozymes, it may be possible to trace evolutionary relationships between populations. Variation in dsRNA between isolates may prove a useful feature to study. Newton *et al.*, (1985) found consistent differences between the dsRNA of isolates of *Puccinia striiformis*.

EXPERIMENTAL WORK PART 2 - Responses of *Pseudocercosporella* to fungicides

6.0 CHEMICAL CONTROL OF EYESPOT

6.1 INTRODUCTION

6.1.1 Control of eyespot

Salt (1955) reported a reduction in eyespot, as well as weed control, after spraying winter wheat with sulphuric acid. Diercks (1964) achieved effective results with the use of calcium cyanamide and mercurial compounds. These compounds are of little use in agriculture because of their high toxicity. Chlormequat reduces eyespot without having a direct effect on the fungus (Slope *et al.*, 1969). Chlormequat, a growth regulant, inhibits cell extension in some plants, particularly wheat and oats; stem length is shortened and thickened and the likelihood of lodging is reduced. Slight control of eyespot is obtained and Diercks (1964) described its successful use in Germany, especially when combined with a fungicide for spring spray applications.

The introduction of systemic fungicides made possible eradication as well as prophylaxis of plant disease. The benzimidazole fungicide, benomyl, was first described in 1968, (Delp and Klopping, 1968) and in 1971, Witchalls and Close reported its successful use in N.Z. against eyespot, with growth of *Pseudocercosporella* being suppressed on media amended with 16 ug a.i. ml^{-1} benomyl. It is a systemic fungicide which moves acropetally through plants and has protectant and eradicant properties. As well as being a broad-spectrum fungicide, it is a mite ovicide yet has the advantage of not being harmful to host tissue. One spray with benomyl of cereals at G.S. 31 (Zadoks *et al.*, 1974) has been recommended for eyespot control (Witchalls and Close, 1971).

Clemons and Sisler (1969) described the formation of a fungitoxic derivative from benomyl after its separation on silica gel. Fifty per cent of the benomyl was found to break down after approximately one hour and the derivative was described as benzimidazole carbamic acid, methyl ester (MBC), also known as carbendazim (Hampel and Locher, 1973). Benomyl was thought to have superior penetration properties to

MBC, however its unstable nature in water suggests that antifungal activity at sites removed from application should be attributed to MBC or a derivative. Undissolved benomyl residues on plant surfaces could maintain an appreciable supply of the compound for considerable periods of time. MBC, itself, can persist on treated mineral soils or plants without modification (Seiler, 1975).

Other fungicides have been evaluated for eyespot control and one of these, prochloraz, was reported by Harris *et al.*, (1979), to be a successful control measure. Glasshouse (Birchmore *et al.*, 1977) and field (Weighton *et al.*, 1977) results have shown that emulsifiable and water dispersible concentrates are the most effective. Reduced eyespot infections have been achieved in the U.K. following field treatment with a mixture of prochloraz and carbendazim (Barnes *et al.*, 1983; Matthews *et al.*, 1985).

Prochloraz is not truly systemic, but is translaminar. It lacks cross-resistance with benzimidazole fungicides and it acts by inhibiting biosynthesis of ergosterol. More specifically, it is thought to inhibit the demethylation of 24-methylenedihydrolanosterol as this ergosterol precursor accumulates in the presence of prochloraz. Chemicals invoking this type of inhibition are more frequently referred to as demethylation inhibitors or DMIs.

DMI fungicides are capable of inhibiting plant growth, probably by the inhibition of gibberellin biosynthesis (Buchenauer, 1977). The demethylation of kaurene during gibberellin biosynthesis has a similar mechanism to the demethylation of 24-methylenedihydrolanosterol.

Prochloraz has an imidazole chemical structure (Table 2.1). Other ergosterol biosynthesis-inhibiting (EBI) fungicides, although having slightly different modes of action, include the triazoles such as triadimefon, triadimenol and propiconazole. Another triazole fungicide is the experimental chemical DPX H6573. It is a systemic chemical with a broad spectrum of activity including reasonable control of eyespot (Fort and Moberg, 1984). As DPX H6573 has not yet been registered for control of eyespot, studies are restricted to laboratory and field trials, where it has been reported to be as

effective as prochloraz for the control of eyespot (Griffin and King, 1985).

6.1.2 Fungicide resistance

6.1.1.1 General fungicide resistance

There is much published literature on the development of resistance to fungicides (Dekker, 1972; Siegel and Sisler 1977; Davidse and de Waard 1984; Dekker and Georgopoulos, 1982) and numerous examples occur in N.Z. (Hartill, 1986).

Some compounds have fungicidal effects on some isolates and fungistatic effects on other isolates of the same population. For the purpose of this study, all compounds capable of causing some reduction in fungal growth shall be called fungicides. A fungal isolate shall be called resistant when a single major gene change results in a complete lack of control by a fungicide. This is signified by no disease control in the field, and in the laboratory, fungal growth occurring to the same extent on fungicide-amended culture medium as it does on unamended media.

For a pathogen population to become fungicide-resistant, resistance alleles must become occurrent in the gene pool of the population and have accumulated to levels preventing adequate control of the population by fungicide treatment. The possession of these alleles must have no deleterious effects on the pathogen or its fitness and they would preferably be maintained within the population during periods between fungicide exposure. Situations where this could occur with ease are those in which the fungicide has a single site of action, whereby a single genetic change, probably arising from mutation, renders an isolate completely resistant. Frequent spraying maintains constant pressures on a fungal population and selects for these resistance genes. Fungal genera with high reproductive capacities are then able to quickly accumulate these genes within the population. Asexual reproduction can result in the production of fungicide-resistant isolines. Enclosed environments in particular, such as those of greenhouse crops, prevent the continued introduction of sensitive alleles.

Early examples of pathogens resistant to benomyl included *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll., cause of powdery mildew of cucurbits, (Schroeder and Provvidenti, 1969) and *Botrytis cinerea* Pers., cause of heart rot of cyclamen (Bollen and Scholten, 1971). In 1985, reduced sensitivity to DPX H6573 was reported in field isolates of *Venturia inaequalis* (Cooke) Winter (Stanis and Jones, 1985). Genetic analysis indicated that the reduced sensitivity was determined by a single nuclear gene. Further studies have found examples of resistance controlled by numerous genes or gene alleles.

Potential problems with EBI fungicides include the decreased sensitivity of *S. fuliginea* to bitertanol, fenarimol and triforine in the Netherlands and Israel (Stanis and Jones, 1985). Multigenic insensitivity is characterised by less complete changes in a pathogen's response to a fungicide, with insensitivity increasing as the pathogen accumulates insensitivity genes. These genes may exist in a wild population before selection pressures have been imposed by chemical treatment rather than accumulating from rare mutations as is the case with resistance, hence development of high levels of insensitivity may be slower than that of single-gene resistance and may, at least initially, go unnoticed. The rate of accumulation depends on the frequency of sexual recombination, the selection pressures imposed and the subsequent rate of asexual reproduction. It would be for this reason that studied examples are fewer and more recent than those of single major gene resistance. The mean response of a population to a fungicide may shift after selection by fungicide treatments. Small shifts would be signified by small changes in EC50 values. Skylakakis (1985) described such responses as quantitative rather than qualitative. In the present study they are described as insensitive.

Dimethirimol was introduced in the Netherlands in 1968 for use against *Sphaerotheca fuliginea* (Elias *et al.*, 1968). It was applied as a suspension in soil and provided continuous plant protection for several weeks. Within a year, results were less satisfactory in some glasshouses, and by 1971, resistant mildew was widespread in the Netherlands (Bent *et al.*, 1974). Dimethirimol and ethirimol, both hydroxypyrimidine fungicides, only affect powdery mildews which are

obligate pathogens, limiting study to the fungus growing on host tissue.

6.1.1.2 Genetic mechanism of benomyl resistance

Heterozygous diploid strains of *Aspergillus nidulans* (Eidam) Wint. may spontaneously segregate into new diploid and occasionally haploid genotypes. When spread on benomyl-amended media *A. nidulans* was inhibited in colony growth and at high concentrations colony size was reduced. Benomyl appeared to induce segregant formation, however, and segregants were predominantly haploid. Sectoring was not induced in haploid strains and no reversions of auxotrophic mutants were noted, suggesting that benomyl does not cause gene mutations (Hastie, 1970; Kappas *et al.*, 1974). Resistant mutants had unaffected growth rates and sporulation, hence microtubules would still form and function normally. If nondisjunction is responsible for sectoring, increased sectoring implies improper functioning of microtubules. Hastie (1970) showed however, that such mutant diploids are unstable. Further evidence that benomyl has an effect on genetic material was provided by Bartels-Schooley and MacNeill (1971), who showed fungitoxicity of benzimidazole fungicides to be enhanced when *in vitro* pH is neutral or alkaline, but reduced in the presence of purines and certain other compounds involved in nucleic acid synthesis. Two levels of benomyl-resistance have been found in *A. nidulans* (Hastie and Georgopoulos, 1971) and crossings demonstrated the two genes responsible to be non-allelic, non-additive and to recombine freely. Benomyl-resistance has also been induced in isolates of *Neurospora crassa* Shear and Dodge by U.V. irradiation of conidia (Borck and Braymer, 1974). Only one level of resistance was found in this organism and expression was dominant, however it was suggested that recessive mutations could be feasible but more difficult to detect. In studies with *Ustilago maydis* (D.C.) Corda and *Saccharomyces cerevisiae* Meyen ex Hansen, mitosis was upset at the time of doublet formation and cell division was not completed (Hammerschlag and Sisler, 1973).

Davidse and Flach (1977) reported that MBC complexes *in vitro* with a protein present in mycelial extracts of fungi. Binding occurred at

4°C. and was competitively-inhibited by oncodazole, colchicine and other microtubule inhibitors but was altered in two different mutants. Fungal tubulin was confirmed to be the binding protein by electrophoresis. Tubulin, a major component of microtubules, is a globular molecule of about 120000 m.w. The molecule is composed of two chemically distinct protomers (α and β-tubulins) which electrophoretic analysis indicates are of equal molecular weight. There is a strong conservation of size, charge and primary sequence between the α and β-tubulins of several diverse species (Bryan, 1974). Mutants resistant to MBC probably have altered tubulin structures with low tubulin affinity for MBC being the likely mechanism. MBC is the first agent found that disrupts fungal microtubules and it is known to also induce microtubule disappearance in mammalian cells. Using genetic analysis, mutations to resistance and to increased sensitivity have been shown to occur in the same gene (Van Tuyl, 1977). If differences are found between primary structures of wild-type and mutant strains, it may be confirmed that this is the same gene that codes for tubulin. The benomyl binding site appeared to be on β-tubulin and at least one of the benomyl-resistance genes must be a structural gene for β-tubulin (Sheir-Neiss *et al.*, 1978). As benzimidazole compounds are selective in their action, tubulin from different sources may have different affinities for them. Decreased binding affinities may not be the only mechanism of resistance. Increased affinities between tubulin and microtubule ends, resulting in more stable microtubules less affected by the depolymerisation action of benzimidazoles, may be another mechanism (Davidse and De Waard, 1984).

6.1.1.3 Cross-resistance

Fungal isolates resistant to a fungicide will more than likely be cross resistant to other fungicides with similar modes of action. This resistance is usually mediated by the same genetic factor (Georgopoulos, 1977), in contrast to multiple resistance which describes different chemical resistances under different genetic control. Isolates demonstrating a high degree of resistance to one fungicide may exhibit an increased sensitivity to another. This negatively correlated cross-resistance is useful in the control of resistant fungi, whereby application of mixtures of these chemicals

maintain control (de Waard, 1984). Fenarimol-resistant isolates of various fungal species displayed a degree of negatively correlated cross-resistance to dodine (de Waard and van Nistelrooy, 1983). Carboxin-resistant *Ustilago maydis* was reported as showing increased sensitivity to antimycin A, in comparison with wild types (Georgopoulos and Sisler, 1970). Fungal isolates of numerous genera, resistant to benzimidazoles have been found to exhibit negatively correlated cross-resistance to the N-phenyl carbamates such as barban, chlorpropham, chlorbufam and MDPC (methyl N-(3,5-dichlorophenyl carbamate) (Kato *et al.*, 1984; Suzuki, 1984). Benomyl-resistant isolates of *Pseudocercosporella* were found to be more sensitive to MDPC than benomyl-sensitive isolates, suggesting either MDPC or a similar compound to be of some use in alleviating the benomyl resistance problem (Bateman *et al.*, 1985).

Isolates of *Pseudocercosporella* tolerant of benomyl have not been reported to ever require the chemical for maintaining optimum growth. Isolates resistant to benomyl to the extent that they become dependent on it have been described for *Botrytis cinerea* (Bollen and Scholten, 1971). Acenaphthene (1,8 ethylenapthylene) has a similar effect to benomyl. Kostoff (1938) described irregularities in mitosis and polyploidy induced by acenaphthene in *Triticum* spp and *Secale cereale* L.. *Penicillium chrysogenum* had been found, in the presence of acenaphthene to non-randomly mutate to become partially dependent upon it (Curtis *et al.*, 1956). The requirement was not complete, but growth and sporulation of acenaphthene-requiring mutants was reduced in the absence of acenaphthene. Production of penicillin by these mutants was higher in the presence of acenaphthene than in its absence, however it was lower than that of the parental strain.

6.1.3 Resistance of *Pseudocercosporella* to fungicides

6.1.3.1 Benomyl

Chidambaram and Bruehl, (1973) stated that 'we know neither the concentration of benomyl within the sprayed plant nor the concentration of benomyl required to prevent development of the pathogen within host plants with varying degrees of resistance.' Their early studies in Washington State, USA, found concentrations of benomyl over the range 0.25 to 0.75 ug a.i. ml^{-1} were required to prevent growth *in vitro*. No benomyl-resistant strains were obtained in laboratory studies either spontaneously or following the use of a mutagen.

It was not until 1985 that resistance was reported at concentrations of 3 ug a.i. ml^{-1} in Pullman, USA (Bruehl *et al.*, 1985). The extent of resistance was unknown, however, as there has been very little use of benomyl in the USA it is perhaps not surprising that there have been no reports of field control failure.

In 1975, Rashid and Schlosser reported the isolation near Gossfelden, FRG of *Pseudocercosporella* isolates resistant to 10 ug a.i. ml^{-1} benomyl, from the stubble of benomyl-treated wheat. There was no apparent reduction in hyphal vigour or sporulation of these isolates. The frequency of resistant spores was considered very low and along with factors including low selection pressures, the limited spread of the fungus, its high reproduction rate and long latent periods field problems with resistance were not considered likely to develop (Horsten and Fehrmann, 1980a,b).

In 1985, Schreiber and Schlesinger reported two levels of benomyl resistance in FRG isolates. Fehrmann (1985) described a change in Northern German populations as more than 50% of sampled sites contained resistant isolates. These sites had usually been sprayed more frequently with benomyl than sites containing sensitive isolates. For that reason, it was suggested that Southern Germany may experience similar changes in resistance frequencies in the future, after frequent exposure to benomyl.

Benomyl was used successfully for a decade in the U.K. until in a 1981 survey, two sites were discovered in England where eyespot was no longer satisfactorily controlled. Isolates taken from these sites were found to grow on benomyl-amended agar. Follow-up surveys in 1983 detected the presence of benomyl-resistant isolates in all major cereal growing areas of England (Brown *et al.*, 1984). By 1985, resistance was becoming a serious threat to the effectiveness of MBC fungicides in the U.K. (Scott and Hollins, 1985). Some form of association between MBC resistance and R-type phenotypes has been suggested (Griffin and Yarham, 1983; Brown *et al.*, 1984; King and Griffin, 1985) although the limited data presently available provide no evidence for genetic linkage (Bateman *et al.*, 1985). The U.K. population is now predominantly R-type and MBC-resistant (Scott and Hollins, 1985; Bateman *et al.*,1986). Hoare *et al.* (1986), reported the artificial inoculation of a wheat field trial with MBC-sensitive R- and W-type spores. After three consecutive years of carbendazim sprays, more than 90% of sampled isolates were MBC-resistant, of which the majority were R-type. It was suggested that MBC fungicides select for both resistance and R-type morphology, but that the two traits are independently selected.

A survey of Southland, N.Z. was undertaken in February, 1984, to determine if benomyl resistance occurs in N.Z. isolates of *Pseudocercosporella* (King *et al.*, 1984). Resistance, at 2 ug a.i. ml^{-1} benomyl, was found in 22 of 131 sampled isolates, present in both barley and wheat crops. Numerous levels of resistance were found among isolates.

6.1.3.2 Prochloraz

Of 674 isolates of *Pseudocercosporella* tested on media containing prochloraz, five showed some growth (Scott and Hollins, 1985) but this growth was described as inconsistent (Scott, *pers. comm.*).

Buchenaeur *et al.*, (1985) induced insensitivity to prochloraz *in vitro* in isolates of *Pseudocercosporella* after treatment of conidia from sensitive isolates with N-methyl-Nl-nitro-N-nitrosoguanidine (NG). Production of conidia and virulence was less in the

induced-insensitive strains however there was no evidence to indicate that there would be a relationship between insensitivity and virulence of wild isolates.

Fuchs and Drandarevski (1976) considered the development of resistance to ergosterol biosynthesis inhibitors to be unlikely because of the role of ergosterol in sporulation.

6.2 *IN VITRO* RESPONSES OF ISOLATES TO BENOMYL

6.2.1 Method

Isolates were tested on PDA amended with benomyl over a range of concentrations; 0.02, 0.2, 2, 20 and 200 ug a.i. ml^{-1}. Each test was duplicated and plates were incubated at 15-18°C for a week. Both unamended PDA and PDA amended with ethanol were used in control plates.

6.2.2 Results

The distribution of sites at which benomyl-resistant isolates were found in Southland is shown in Fig. 6.1 and benomyl resistance data for the three seasons is in Table 6.1.

Fig. 6.1 MAP OF SOUTHLAND SHOWING SITES WHERE BENOMYL-RESISTANCE WAS DETECTED

Table 6.1

Percent benomyl-resistant isolates collected from wheat and barley crops in annual surveys.

SEASON	%benomyl-resistant isolates on : (*sample size) WHEAT	BARLEY	TOTAL
1983/84	15.39% (52*)	16.66% (72)	15.87% (124)
1984/85	63.33% (11)	17.65% (17)	35.71% (28)
1985/86	12.77% (53)	57.14% (7)	17.74% (60)

The number of fields sampled in Canterbury were too few to give a true representation of the area. Isolates were obtained from two South Canterbury sites and one site in North Canterbury. No benomyl resistance was found.

There are no apparent trends in levels of resistance of isolates over the three years of surveys, although highly resistant (i.e. resistant at 200 ug a.i. ml^{-1}) isolates were obtained from sites which had had numerous benomyl sprays. Low-level resistant isolates predominated.

Numbers of isolates resistant to benomyl at tested concentrations are listed in Table 6.2. Resistance at 0.002 and sometimes 0.02 ug a.i. ml^{-1} was difficult to determine. Colonies frequently did not grow out from hyphal plugs placed on the medium, however, the isolates remained dormant. The hyphal plugs were frequently characterised by pink growth and production of stromatic cells. When subcultured onto unamended PDA, hyphal colonies grew at normal rates, however the mycelium was abnormal, being pale and loose.

Table 6.2

No. isolates resistant to benomyl at different concentrations

Season	Res. at 0.002	0.02	0.2	2.0	20	200 ug ml^{-1} benomyl
1983/84	-	0	2 (20%)	3 (30%)	0 (0%)	5 (50%)
1984/85	5(50%) 8%)	2(20%)	-	-	0 (0%)	3 (30%)
1985/86	-	34(56.66%)	16(26.66%)	5(8.33%))	0 (0%)	5(8.33%))

Growth of isolates on a range of benomyl concentrations is shown in Figs. 6.2 and 6.3.

Sample sizes were too small to determine a definite correlation between the probability of detecting resistance and sites with a history of benomyl spraying. Resistance is occurrent in some unsprayed sites e.g. field 36 (1985/86) was a first-year wheat.

Fig. 6.2 Growth of isolates on control, 0.2BEN and 2BEN PDA

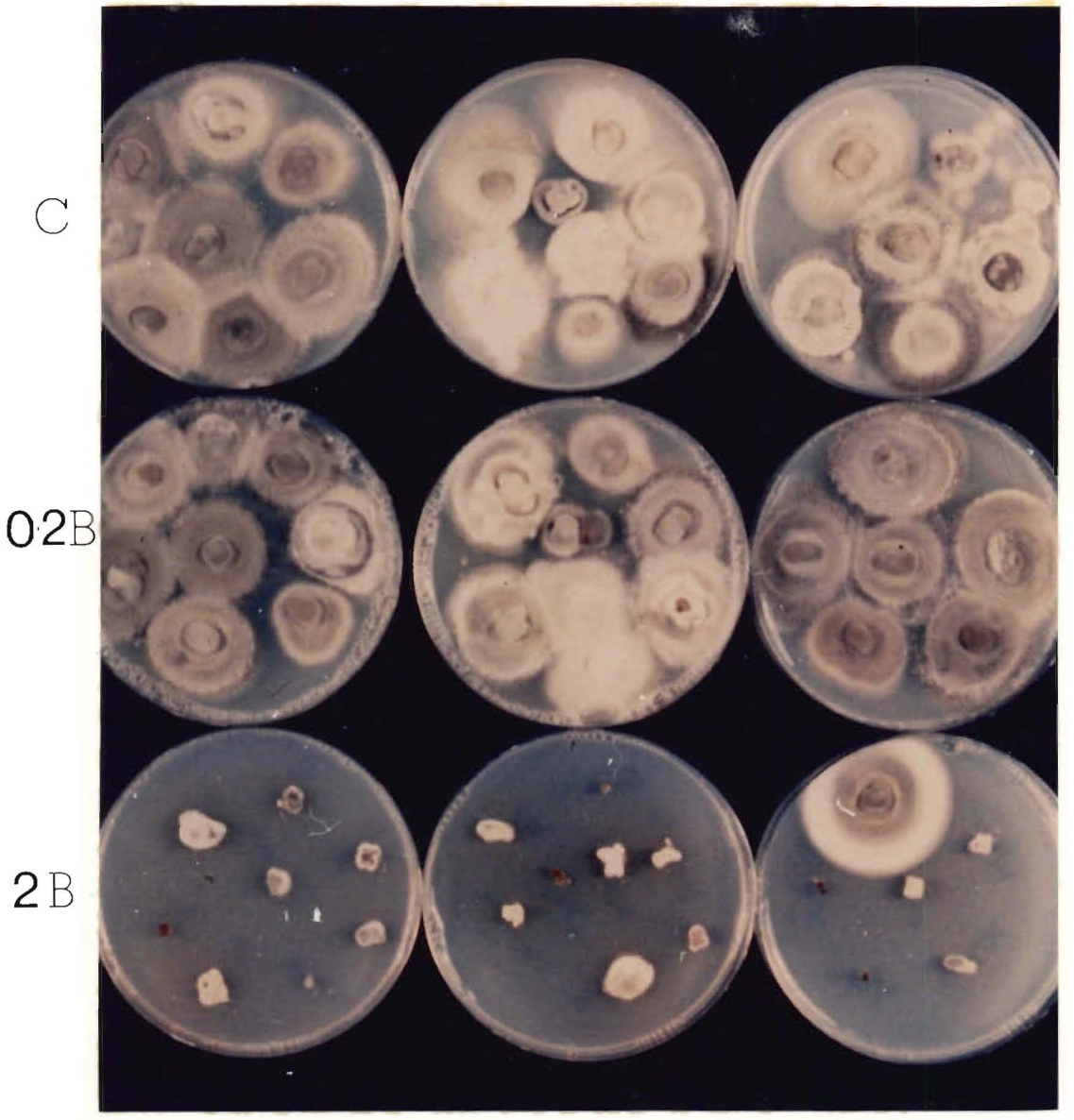
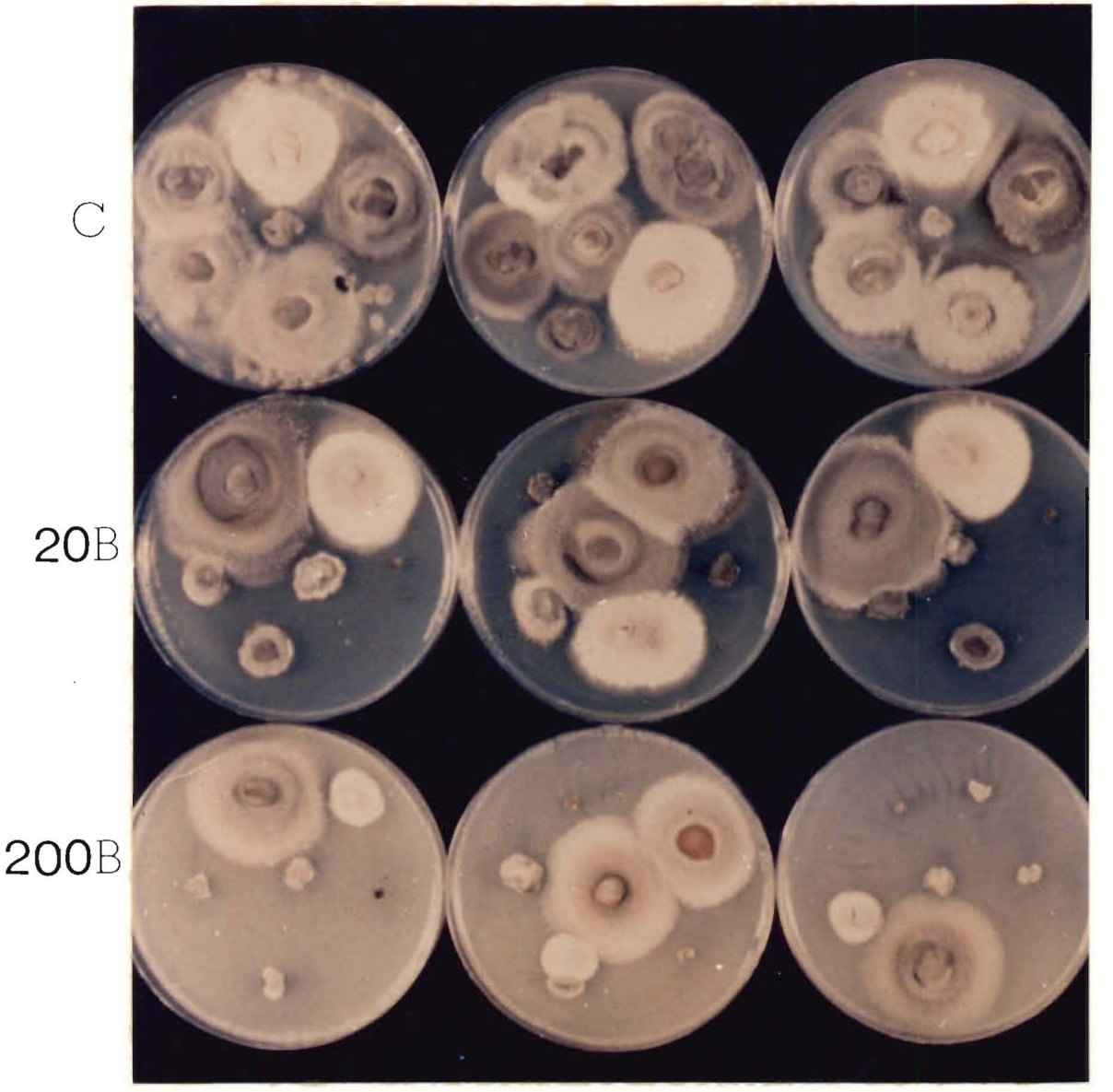


Fig. 6.3 Growth of isolates on control, 20BEN and 200BEN PDA



6.2.3 Discussion

Isolates were obtained from hyphae originating from stem material collected during the surveys and these were subcultured onto media amended with fungicide. This was the quickest way to transfer isolates to *in vitro* fungitoxicity tests and did not select against mutants deficient in sporulation. Host lesions are not always initiated from a single spore, and therefore presumed homokaryotic, but could also originate from a combination of two or more karyons growing together. If anastomosis occurred between genetically different mycelia, the resulting isolate would be heterokaryotic. Davies and Jones (1970) provided evidence for a diploid 'hybrid' resulting from hyphal and subsequent nuclear fusion.

Criticisms have been made of testing mass-mycelial isolates, including the overestimation of resistance frequencies (Fehrmann, 1983). Evaluation of the two techniques has been summarised by King and Griffin (1985) to show they give similar estimations of the extent of resistance. Only resistant conidia have been obtained from isolates from stems in which resistance is present. Fungitoxicity tests demonstrated the number of lesions containing benomyl-sensitive or resistant *Pseudocercosporella* nuclei. It is these nuclei which are removed or selected under a spray regime which is reflected in the build-up of particular population components in the following season.

Frequently, after the addition of fungicide to media, isolates have been reported to show increased growth rates. This phenomenon has been suggested by Brown *et al.*, (1984) to be a result of an increase in linear growth rate and a reduction in hyphal density. The use of EC50 values in categorising isolates may perhaps mask trends which could reflect what is happening in the field. Such changes in growth type could be used to exploit a changed environment. The SF or FE growth types of *Pseudocercosporella* immediately complicates such tests. EC50 values were hence not determined. Fletcher and Yarham (1976) suggested that isolate resistance may be overestimated by the testing of various concentrations of benomyl-amended agar, as benomyl is only partially soluble in water. Workers using carbendazim (e.g. Bateman *et al.*, 1986) however, obtained similar results.

The MBC situation appears stable in N.Z., with the number of resistant isolates remaining at between 15 and 20% of the population. This is supported by Birchmore *et al.* (in press) which reports a similar N.Z. survey undertaken in the 1985/86 season. Their figure for total resistance is 19%.

Following reports in the U.K. (Rashid and Schlosser 1977; Brown *et al.*, 1984), resistance has now been found at N.Z. sites where no benomyl has been used.

Benomyl has been used by cereal growers in N.Z. since 1971 (Witchalls and Close, 1971), and yet resistance has not developed in *Pseudocercosporella*, to an uncontrollable level. Reasons for the prediction that benomyl resistance would not become a major problem in West Germany, included the low selection pressures combined with the small proportions of resistance already present within the population (Horsten and Fehrmann, 1980a,b). These reasons could perhaps be applied to the N.Z. population with a similar prediction. Intensive cropping and spraying practices place strong selection pressures on a fungal population and to prevent the development of benomyl resistance problems, selection pressures must be minimised. At sites with levels of resistance of around 20%, a dramatic increase would be expected to occur with intensive use of benomyl. It would appear that the exponential increase in proportions of a resistant population has a threshold level, at which control failure will occur and complete resistance develop. King and Griffin (1985) suggest the threshold level could be lower than 30%, based on ADAS trials in 1982 and 1983. Resistance is present in N.Z., but at levels low enough to be contained by short crop rotations with few sites having more than three years of cereals. The sowing of winter wheat has not been encouraged and benomyl sprays are not normally applied to first-year crops. These strategies must be prolonging the useful life of benomyl for eyespot control in New Zealand.

6.3 ADDITIONAL FUNGICIDE EXPERIMENTS

6.3.1 Methods

6.3.1.1. Shaking liquid-culture

The addition of benomyl to agar poses the problem of solubility (Fletcher and Yarham, 1976) and even distribution of low concentrations. Resistance/sensitivity of fungal isolates was therefore also determined using shaking liquid-cultures.

Potato dextrose broth (50ml) was added to each of 25 125ml flasks and then amended with one of five concentrations of benomyl (0, 0.2, 2, 20 and 200 ug a.i. ml^{-1}). Five flasks were amended per treatment. Within each treatment, two flasks were inoculated with an isolate (GHJA1/4/3) resistant to benomyl at 20 ug a.i. ml^{-1} (as determined on agar - BI). Two other flasks were inoculated with a 'high-level' resistant isolate (GHJA1/5/1) resistant to benomyl at 200 ug a.i. ml^{-1} (as determined on agar - BH). A fifth flask was inoculated with an isolate (86/36/11) sensitive to benomyl at 0.2 ug a.i. ml^{-1} (as determined on agar - BS), as a control. Inoculum consisted of two hyphal plugs (5mm) taken from the margin of actively-growing colonies on unamended PDA. These subcultures had never been exposed to benomyl *in vitro*. After 30 days on an orbital shaker, mycelium was filtered and dried.
see p. 20, lines 12-15

6.3.1.2. Responses of isolates of *Pseudocercosporella* to MDPC.

Isolates, 85/11/1 (benomyl-sensitive) and 85/8/2 (benomyl-resistant to 200 ug a.i. ml^{-1}), were tested for their responses to MDPC.

6.3.1.3. Effect of acenaphthene on colonies of *Pseudocercosporella*

Isolates of *Pseudocercosporella* do not appear to become dependent on benomyl in any way, such as could result from induced mutation. To investigate the potential of this happening in response to other chemicals with similar sites of action, isolates of *Pseudocercosporella* (85/1/1 and 85/5/6) were tested for response to acenaphthene, a chemical known to upset mitosis in plants.

6.3.2 Results

6.3.2.1. Shaking liquid-culture

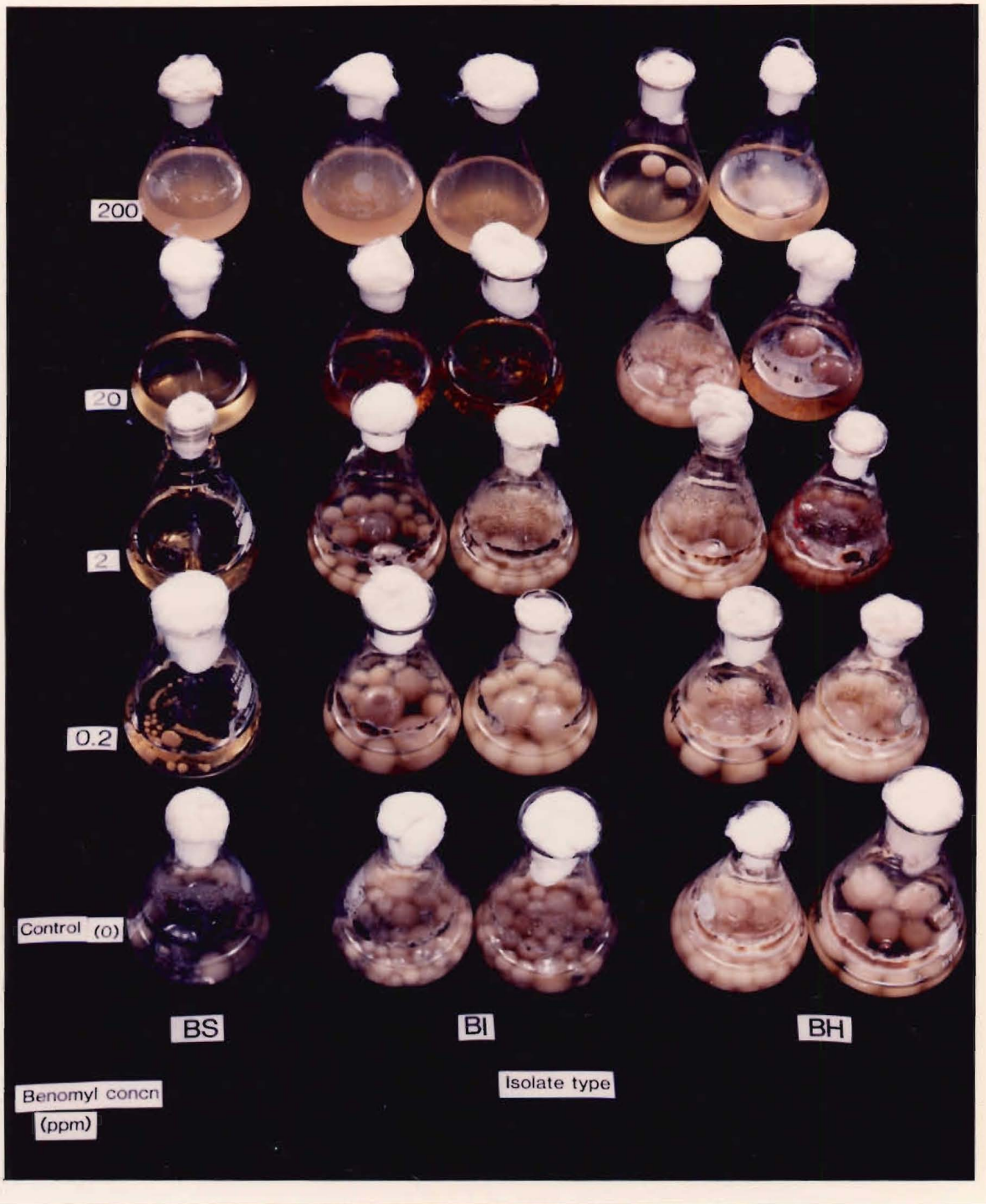
Results showed that benomyl-resistant isolates, as described by responses on agar medium, produced the same growth in shaking liquid-culture amended with benomyl (Table 6.3, Fig. 6.4). Combined with the reproducibility of results in replicated agar tests, this supports the use of agar as an adequate test for determining relative resistance/susceptibilities of different isolates to benomyl.

Table 6.7

Dry weight (g) of isolates tested in shaking liquid culture

	ISOLATE		
Treatment	BS	BI	BH
Control	0.47	0.52	0.52
0.2BEN	0.09	0.44	0.51
2.0BEN	0.05	0.43	0.52
20BEN	0.05	0.17	0.32
200BEN	0.02	0.04	0.09

Fig. 6.4 Growth of isolates within shaking liquid-culture amended with benomyl



6.3.2.2. Responses of isolates of *Pseudocercosporella* to MDPC.

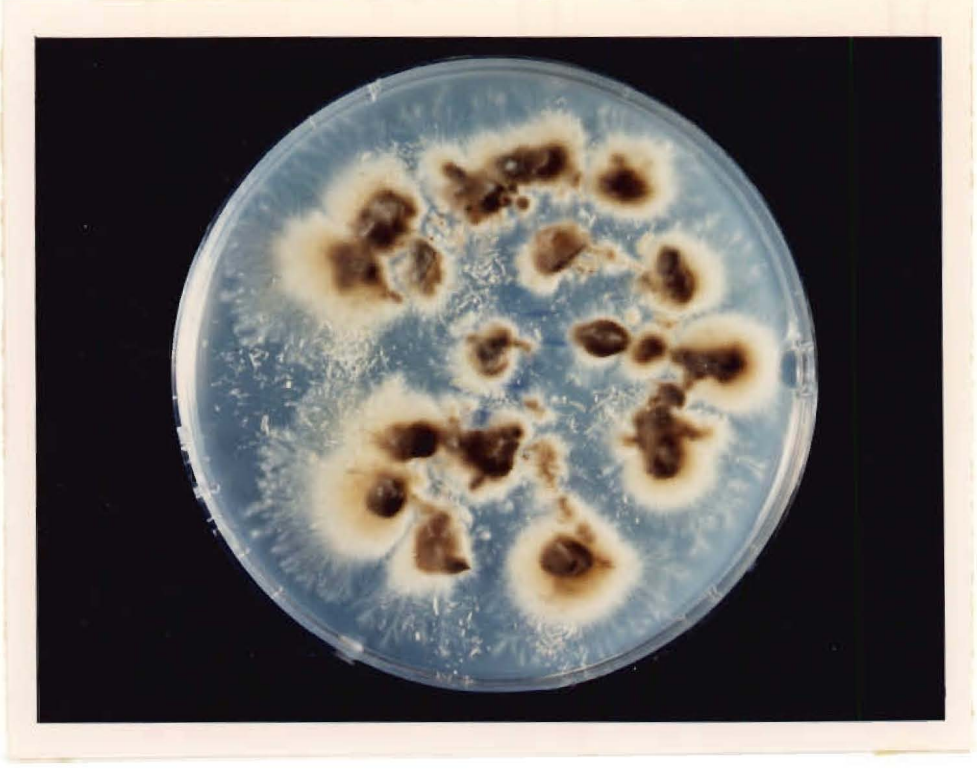
Both the benomyl-resistant and sensitive isolates produced growth on all rates of MDPC-amended PDA, as on control plates. MDPC would have no practical use in controlling benomyl-resistant *Pseudocercosporella* isolates, therefore no further work on this was done.

6.3.2.3. Effects of acenaphthene on colonies of *Pseudocercosporella*

The isolates grew at a normal rate but were unusual in colour. Some growth of isolate 85/1/1 extended through the crystals (Fig. 6.5). After hyphal plugs were removed from the margins of colonies and plated onto fresh PDA, growth continued but was abnormal.

Fig. 6.5 Growth of isolates on agar sprinkled with acenaphthene crystals

85/1/1



85/5/6

6.4 RESPONSES OF ISOLATES OF *PSEUDOCERCOSPORELLA* TO PROCHLORAZ

6.4.1 Introduction

In 1983/84, 20 isolates of *Pseudocercosporella* (see Appendix 1) collected from Southland cereal crops, were tested on prochloraz-amended agar and found to be sensitive at 2 ug a.i. ml^{-1}. As Scott and Hollins (1985) reported that some growth of U.K. isolates occurred on prochloraz-amended medium, further screening of N.Z. isolates on a range of concentrations of prochloraz was required.

6.4.2 Experimental procedures

6.4.2.1. Control test using the prochloraz-sensitive fungus *Rhynchosporium secalis* - to determine the effectiveness of the active ingredient when incorporated in agar medium.

Spore suspensions from seven actively-growing isolates of *R. secalis* were prepared for application to treatment plates using the method of Hollomon (1984). Each isolate was transferred with a sterile inoculating loop (one loopful) from the YMA plates to separate McCartney bottles, each containing 9ml sterile, distilled water. These were shaken until suspensions were uniform. A loopful of each suspension was then streaked across individual 2PRO PDA and PDA plates. Plates were maintained in an 15°C incubator for 14 days.

6.4.2.2. Responses of *Pseudocercosporella* isolates to prochloraz

Thirty one isolates collected during the 1984/85 season were tested on 0.2, 2, 20 and 200 ug a.i. ml^{-1} (King *et al.*, 1986). Following the collection of isolates in 1985/86, testing of sensitivity was continued. Eight isolates of both mycelial and conidial origin, were screened intensively with at least 30 hyphal plugs from each isolate being placed on 2PRO PDA. Hyphal plugs were removed from colonies on PDA with a scalpel instead of a cork borer, so that test plugs were as small as possible. Plugs were removed from both the colony edges and from within colonies of isolates F64 1 and F12 2. Plates were left for 14 days.

6.4.2.3. Determination of radial mycelial growth EC50 values*

Isolates K15 1 and 85/1/1 were grown on 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 PRO PDA. Two perpendicular colony diameters were measured for every colony after 14, 17, 20, 23, 26 and 29 days.

*EC50 values are the effective chemical concentrations required to inhibit colony growth by 50%

6.4.2.4. Responses of isolates to benomyl and prochloraz applied as a mixture in PDA.

Four isolates (85/6/4, K16 4, K15 1, 85/1/1) were tested for their responses to a mixture of benomyl and prochloraz (each at 2ug a.i. ml^{-1}) and compared with their responses to these fungicides individually.

Growth was noted as present or absent for each isolate after five days.

6.4.2.5. Responses of isolates to four concentrations of DPX H6573

Five isolates, 85/2/1, 85/3/1, 85/8/1, 85/4/2 and 85/6/4, were tested on 0.2, 2.0, 20 and 200 DPX PDA. Growth was measured for each colony as the mean of two perpendicular colony diameters after 22 days.

6.4.2.6. Sporulation in the presence of prochloraz.

a) Hyphal plugs of different isolates were placed onto 2PRO DWA and DWA plates. The plates were incubated at 5°C under U.V. light for 18 days and then viewed under an inverted microscope for the detection of conidia.

b) One hyphal plug (5mm) of the mycelial isolate 86/36/9 (FE), was placed in each of forty 5cm petri dishes containing 2PRO DWA. One plug was also placed in each of two 5cm control petri dishes containing DWA, and ten plugs were placed in a 9cm petri dish containing DWA. The procedure was repeated for 86/86/6 (FE) and 86/78/18. The plates were incubated at 5°C under near-UV light (350nm) for 14 days, after which time they were viewed under an inverted microscope and assessed for the presence of conidia.

6.4.2.7. Stability of prochloraz-insensitivity

Isolate 86/1SC/2a* growing on PDA (plate a) was subcultured onto 1PRO PDA (plate b) and after a month, 25/54 plugs had grown into colonies.

* originating from a single conidium

1. Fifty four plugs were removed from growing colonies on plate a, and placed on a fresh 1PRO PDA plate for 23 days.

2. Twenty of the plugs which had not grown on plate b were removed and placed on PDA (test plate c) for 23 days.

3. Fifty one plugs were removed from plate a and placed on 2PRO PDA for 23 days.

6.4.2.8. Stability of prochloraz-sensitivity

Twenty six hyphal plugs were removed from the growing margins of the 'prochloraz-sensitive' colonies growing on test plate c. These plugs were placed on a fresh 1PRO PDA plate and left for 23 days at 15°C.

6.4.3 Results

6.4.3.1. Control test using the prochloraz-sensitive fungus *Rhynchosporium secalis*

No isolates of *R. secalis* which produced growth on PDA grew on 2PRO PDA.

6.4.3.2. Responses of *Pseudocercosporella* to prochloraz

Of the 31 isolates collected during 1984/85, 26 showed some growth on 2PRO PDA (Table 6.4) and 18 of these (from both barley and wheat) grew at concentrations > or = 20 ug a.i. ml^{-1}. Of the 26 prochloraz-insensitive isolates, two were also resistant to benomyl at 200 ug a.i. ml^{-1} (King *et al.*, 1986).

Table 6.4 *In vitro* responses of *Pseudocercosporella* to prochloraz at 2 ug a.i. ml^{-1} (1984/85 survey)

Isolate	Isolated from:		
	Wheat	Barley	Total
Sensitive	2(10%)	3(30%)	5(16%)
Insensitive	19(90%)	7(70%)	26(84%)

Numbers of plugs, from the 1985/86 sample, growing on prochloraz, varied for each isolate, but was never greater than half the number plated (Table 6.5) The German isolate of *P. aestiva* (G64344), produced growth on prochloraz similar to that on PDA.

Table 6.5 Proportion of hyphal plugs producing growth on 2 PRO PDA

At 2 ug a.i. ml^{-1} prochloraz:-		Proportions of hyphal plugs producing hyphal growth per plate (%) :-	
a) mass-mycelial isolates			
86/86/9	5/29, 6/29,	= 11/58	(19.0%)
86/78/18	3/32, 3/12, 9/18, 7/31	= 22/93	(23.7%)
86/86/6	3/36, 6/30, 6/32, 1/40	= 16/138	(11.6%)
86/86/16	7/37, 9/31	= 16/68	(23.5%)
86/86/35	3/24, 1/24	= 4/48	(8.3%)
b) conidial isolates			
86/36/6y	9/22, 11/18, 15/17, 13/17	= 20/40	(50.0%)
86/86/18d	4/27, 9/20, 4/18, 9/15	= 13/47	(27.7%)
86/86/18a	1/18, 0/15	= 1/33	(3.0%)
c) German isolates			
P. herpotrichoides var. *herpotrichoides* (curved conidia)		(G63975) 7/22	(31.8%)
P. herpotrichoides var. *herpotrichoides* (straight conidia)		(G63978) 2/30	(6.7%)
P. anguioides		(G63996) 3/30	(10.0%)
P. herpotrichoides var. *acuformis*		(G63998) 0/31	(0.0%)
P. aestiva		(G64344) 35/35	(100.0%)

Growth of *Pseudocercosporella* hyphae after 4 days on 2PRO PDA is shown in Fig 6.6. Variation in growth between plugs is shown in Fig. 6.7. Growth of the different *Pseudocercosporella* species on 2PRO PDA is shown in Fig. 6.8.

There were no differences in growth patterns between plugs taken from either the colony edges or from within colonies of isolates F64 1 and F12 2.

Isolates K27 4 and 85/6/4 were noted as growing on 200 ug a.i. ml^{-1} prochloraz and K16 4, 86/2SC/7 and GHR58/D grew at 20 ug a.i. ml^{-1}. The same pattern of growth was produced as on 2 ug a.i. ml^{-1} prochloraz except fewer plugs produced growth.

Fig. 6.6 Initial hyphal growth of isolates subcultured onto PRO PDA

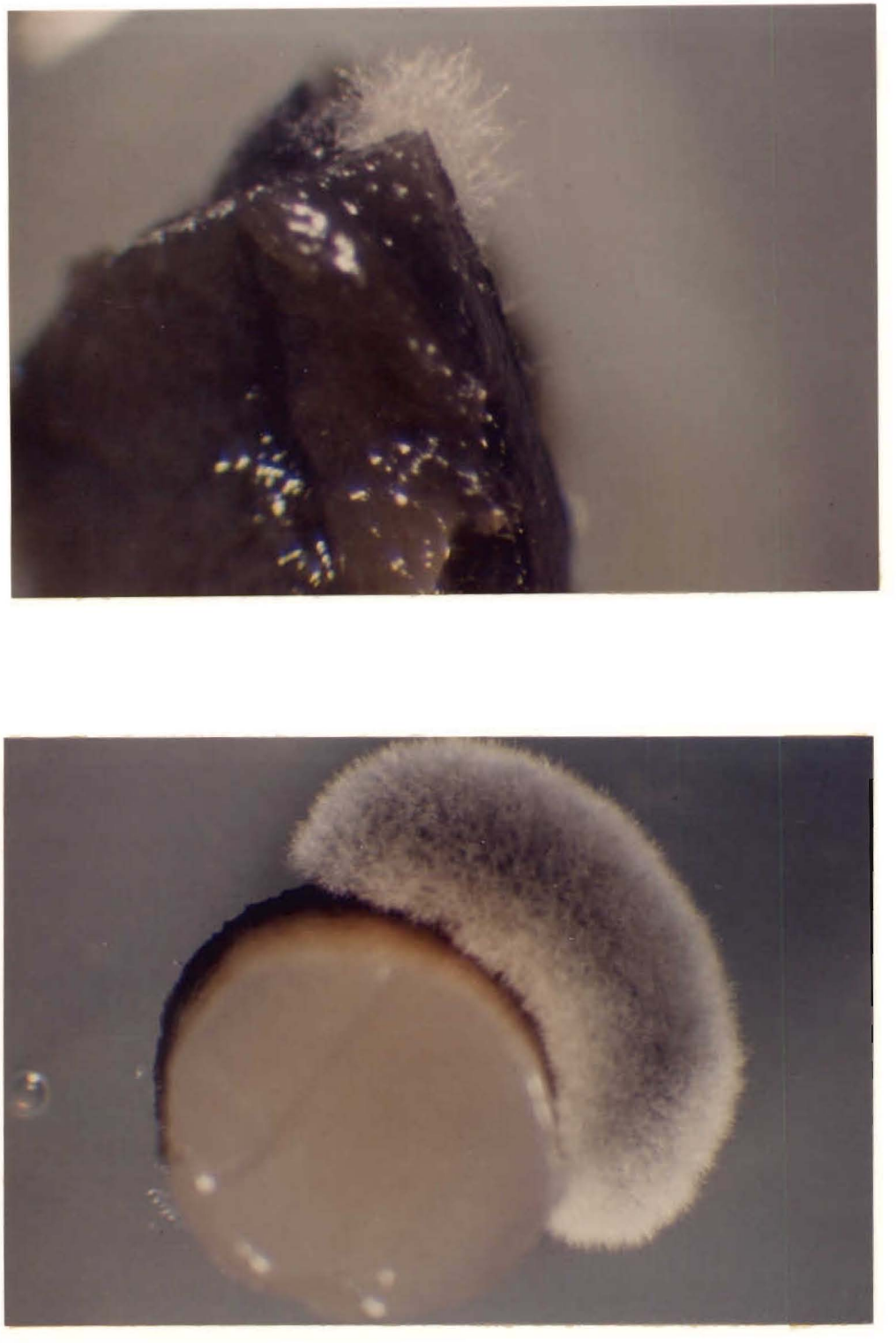


Fig. 6.7Hyphal growth on 2PRO PDA, showing the variation between hyphal plugs from single isolates.

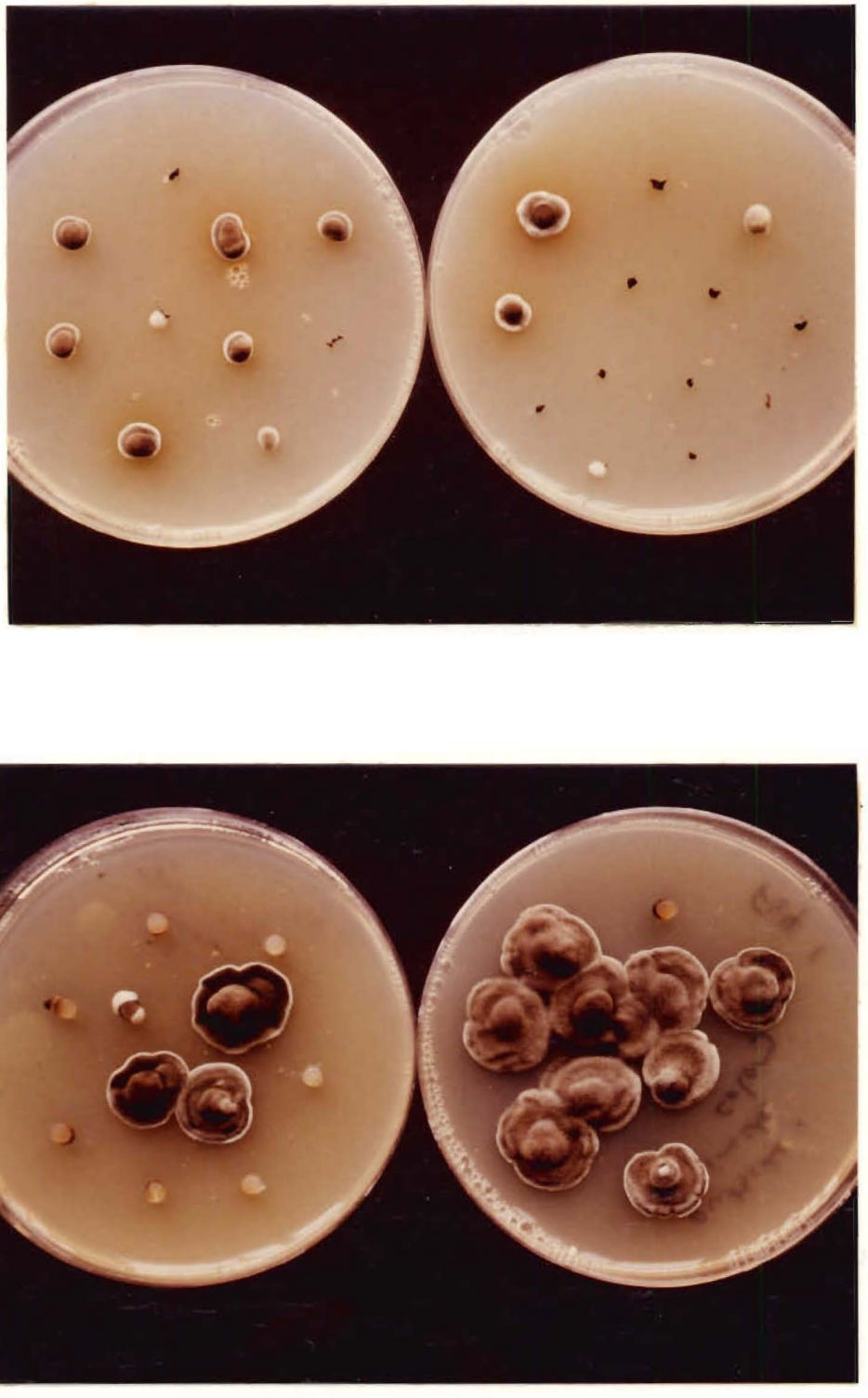
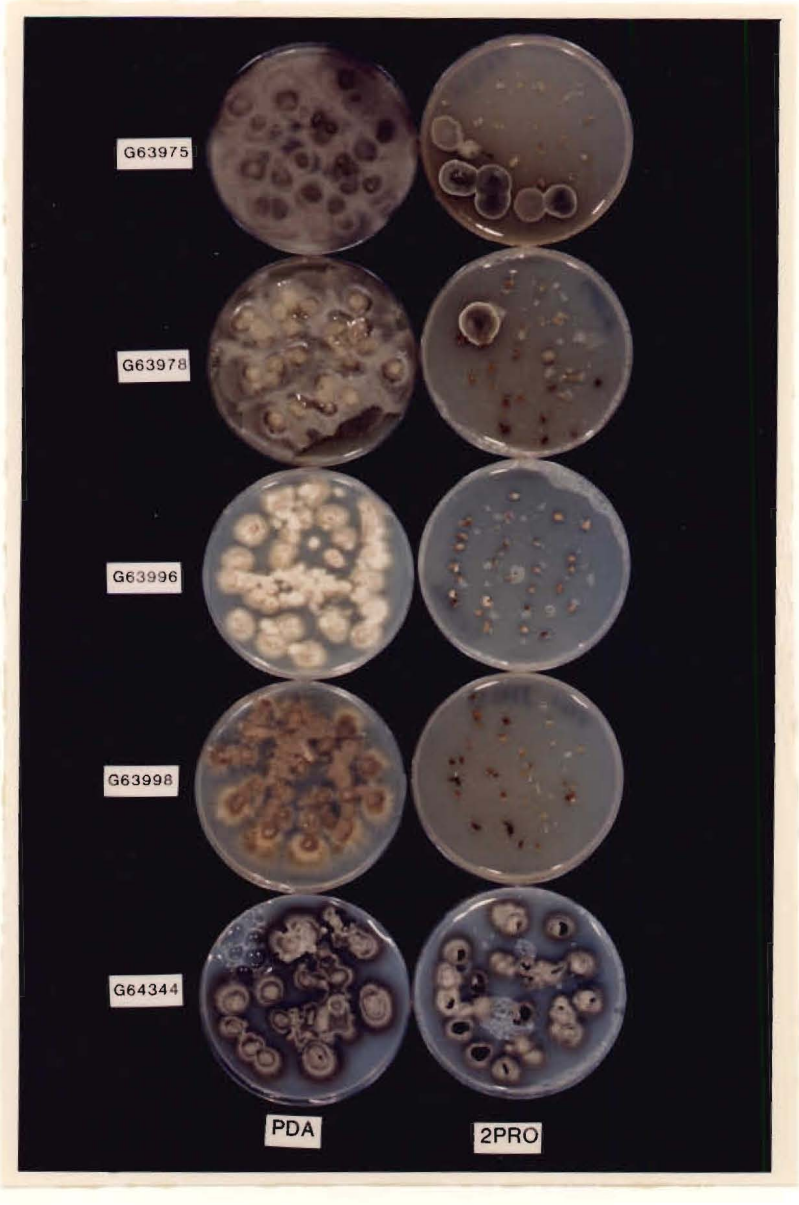


Fig. 6.8 Growth of German isolates on PDA and 2PRO PDA



G63975 - *P. herpotrichoides* var. *herpotrichoides* (curved conidia)
G63978 - *P. herpotrichoides* var. *herpotrichoides* (straight conidia)
G63996 - *P. anguioides*
G63998 - *P. herpotrichoides* var. *acuformis*
G64344 - *P. aestiva*

6.4.3.3 Determination of radial mycelial growth EC50 values

Growth of isolates was constant for each concentration of prochloraz tested, however growth decreased as the concentration of prochloraz was increased (Fig. 6.9). After 29 days, growth of each isolate on different concentrations of prochloraz was described as a percentage of the control (Table 6.6). A plot of these figures (Fig. 6.10), gave the EC50 values to be between 0.1 and 0.5 ug a.i. ml^{-1} prochloraz. The growth of 85/1/1 reached a constant rate, which was maintained at the highest tested concentration (10 ug a.i. ml^{-1}) of prochloraz. Growth of K15 1 was reduced to zero at a concentration of prochloraz between 5 and 10 ug a.i. ml^{-1}. The two isolates showed markedly different levels of insensitivity to prochloraz, even though their EC50 values were similar.

Figure 6.9 Isolates 85/1/1 and K15 1 growing on

(a) PDA
(b) 0.1 PRO PDA
(c) 0.5 PRO PDA
(d) 1PRO PDA
(e) 2PRO PDA
(f) 5PRO PDA
(g) 10PRO PDA

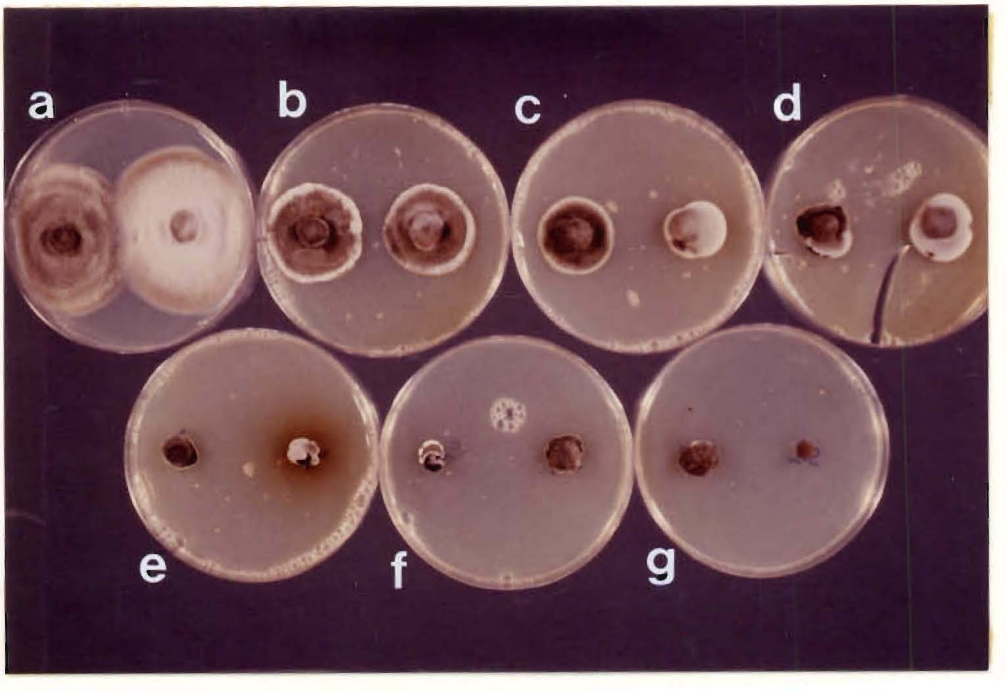


Table 6.6 Colony diameters of 2 *Pseudocercosporella* isolates on agar amended with prochloraz, over 29 days. Each value is a mean of two perpendicular colony diameters (in mm).

4/8 Isolate		Prochloraz concentration µg/ml 0	0.1	0.5	1.0	2.0	5.0	10.0
85/1/1	A	25.00	12.75	12.75	11.00	9.50	2.25	10.00
85/1/1	B	24.50	18.00	12.25	13.00	11.00	11.00	11.75
K15 1	A	24.25	14.50	9.00	13.00	0.00	10.25	0.00
K15 1	B	23.50	15.00	0.00	11.50	11.00	10.00	0.00
7/8 Isolate		Prochloraz concentration 0	0.1	0.5	1.0	2.0	5.0	10.0
85/1/1	A	31.50	22.00	16.00	11.50	9.50	4.00	11.50
85/1/1	B	30.50	22.00	16.00	14.50	12.50	11.50	13.00
K15 1	A	32.50	18.50	11.00	15.50	trace	11.00	0.00
K15 1	B	31.00	18.00	0.00	13.50	10.75	11.00	0.00
10/8 Isolate		Prochloraz concentration 0	0.1	0.5	1.0	2.0	5.0	10.0
85/1/1	A	36.50	25.50	18.50	10.50	11.00	7.00	11.50
85/1/1	B	36.00	26.00	19.00	16.00	14.25	12.50	13.00
K15 1	A	40.00	22.50	13.00	17.00	trace	11.50	0.00
K15 1	B	37.75	22.50	0.00	16.00	12.50	12.50	0.00
13/8 Isolate		Prochloraz concentration 0	0.1	0.5	1.0	2.0	5.0	10.0
85/1/1	A	46.25	29.00	21.50	15.00	11.00	8.00	12.50
85/1/1	B	42.00	29.50	21.50	16.50	14.00	14.00	14.50
K15 1	A	46.00	26.50	15.00	19.50	3.00	12.00	0.00
K15 1	B	44.00	26.50	0.00	-	14.25	13.50	0.00

16/8 Isolate		Prochloraz concentration 0	0.1	0.5	1.0	2.0	5.0	10.0
85/1/1	A	49.00*	32.00	24.00	15.50	12.00	8.00	13.00
85/1/1	B	49.00*	33.50	24.00	18.50	15.00	12.50	13.00
K15 1	A	52.00*	29.50	17.00	22.50	8.50	12.50	0.00
K15 1	B	51.00*	30.00	0.00	20.50	15.00	15.00	0.00

19/8 Isolate		Prochloraz concentration 0	0.1	0.5	1.0	2.0	5.0	10.0
85/1/1	A	55.00*	34.50	27.00	18.00	12.00	8.50	14.00
85/1/1	B	53.00*	36.00	27.50	20.50	16.00	14.00	15.50
K15 1	A	60.00*	32.50	20.50	24.00	10.50	14.00	0.00
K15 1	B	58.00*	33.50	0.00	23.00	17.50	18.00	0.00

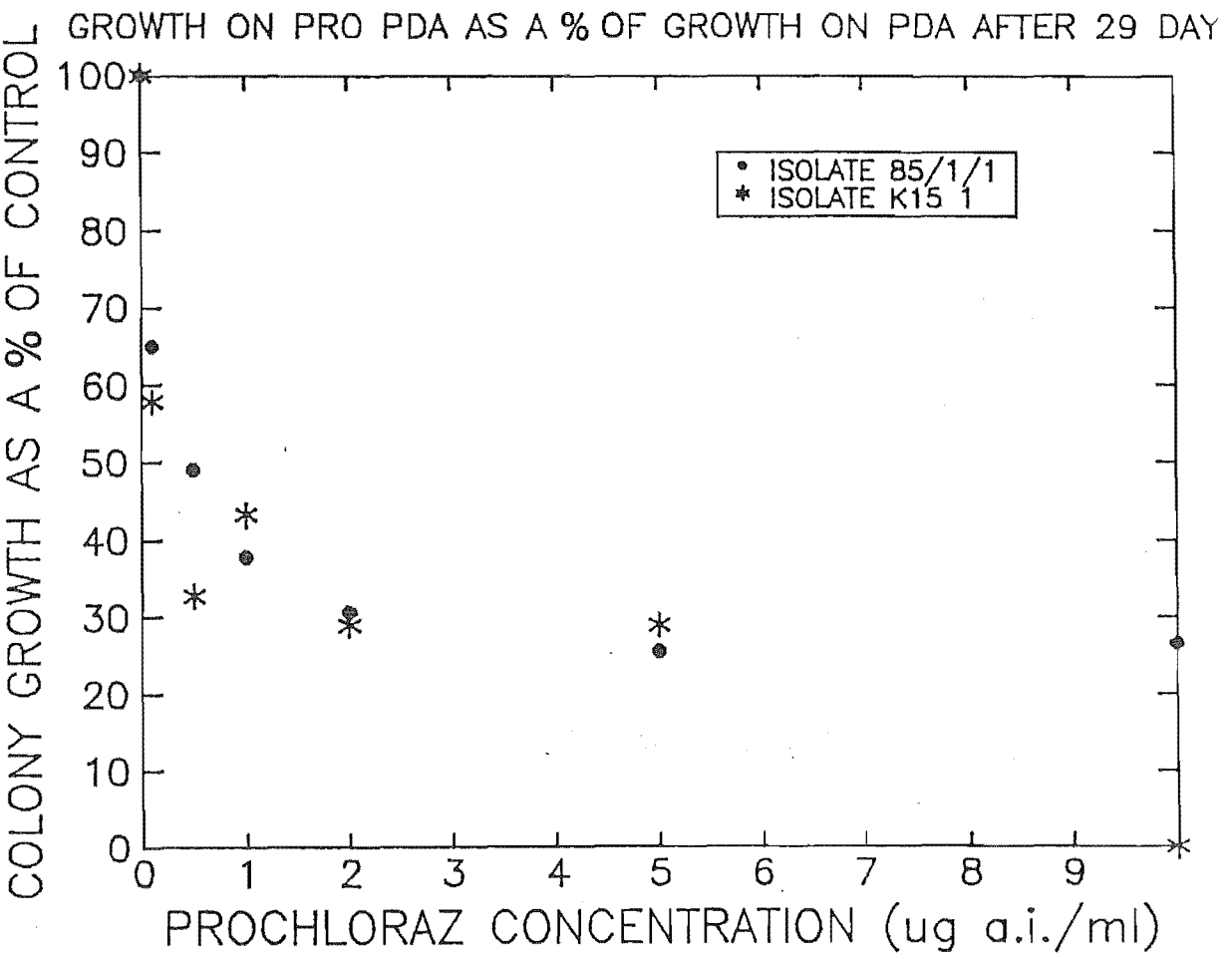
Table 6.7 Colony diameters of 2 *Pseudocercosporella* isolates on agar amended with prochloraz , as a percentage of their diameters on unamended agar, after 29 days.

Isolate		Prochloraz concentration (ug/ml) 0	0.1	0.5	1	2	5	10	EC50
85/1/1	A	100.00	65.00	48.98	37.80	30.60	25.51	26.53	0.46
85/1/1	B	100.00	68.40	48.98	31.60	24.50	16.33	26.53	0.46
K15 1	A	100.00	57.80	32.69	43.27	28.90	28.90	0.00	0.20
K15 1	B	100.00	57.70	0.00	40.20	16.66	24.50	0.00	0.20

A/B = Replicates

* = a single colony diameter measurement

Fig. 6.10


GROWTH ON PRO PDA AS A % OF GROWTH ON PDA AFTER 29 DAY
COLONY GROWTH AS A % OF CONTROL
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
PROCHLORAZ CONCENTRATION (ug a.i./ml)
ISOLATE 85/1/1
ISOLATE K15 1

6.4.3.4. Responses of *Pseudocercosporella* isolates to benomyl and prochloraz applied as a mixture in PDA

Table 6.8 Presence or absence of growth from subcultured hyphal plugs of *Pseudocercosporella* isolates on prochloraz and/or benomyl-amended agar.

Treatment		PDA	2PRO PDA	2BEN PDA	2PRO + 2BEN PDA
Isolate					
K15 1	(BSPI)	+	+	-	-
85/1/1	(BRPI)	+	+	+	+
85/6/1	(BSPI)	+	+	-	-
K16 4	(BSPI)	+	+	-	-

+ = growth
- = no growth

The isolates responded to the mixture of fungicides as was expected from their responses to the individual treatments (Table 6.7). There were no interactive effects.

6.4.3.5. Responses of isolates to four concentrations of DPX H6573

Growth of hyphae from DPX PDA was similar to that from PRO PDA, in that it originated from small points on a hyphal plug and not all plugs from an isolate produced growth.

None of the five isolates tested grew on PDA amended with 20 ug a.i. ml^{-1} DPX H6573 and isolate 85/8/1 was the only isolate which grew at 2 ug a.i. ml^{-1} DPX H6573. The EC50 value was generally less than 0.2 ug a.i. ml^{-1}.

Table 6.9 Colony diameters of 5 *Pseudocercosporella* isolates on agar amended with DPX H6573, after 22 days. Each value is a mean of two perpendicular colony diameters (in cm).

Isolates		0	0.2	2	20	200
85/2/1	A*	3.7	0.6	0	0	0
	B		1.3	0	0	0
85/3/1	A	3.4	0.7	0	0	0
	B		1.4	0	0	0
85/8/1	A	3.2	1.5	-	0	0
	B		1.2	1.1	0	0
85/4/2	A	2.8	1.3	0	0	0
	B		0.9	0	0	0
85/6/4	A	3.5	0.8	0	0	0
	B		0.8	0	0	0

Table 6.10 Colony diameters of 5 *Pseudocercosporella* isolates on agar amended with DPX H6573, as a percentage of their diameters on unamended agar, after 22 days

Isolates		0	0.2	2	20	200
85/2/1	A*	100	16.20	0	0	0
	B		35.14	0	0	0
85/3/1	A	100	20.60	0	0	0
	B		41.18	0	0	0
85/8/1	A	100	46.80	-	0	0
	B		37.50	34.9	0	0
85/4/2	A	100	46.40	0	0	0
	B		32.10	0	0	0
85/6/4	A	100	22.90	0	0	0
	B		22.90	0	0	0

*A/B = replicates

6.4.3.6. Sporulation in the presence of prochloraz

a) All isolates sporulated on DWA. Isolates 86/2SC/11 and 86/86/18 did not sporulate on 2PRO DWA. Sporulation occurred in both replicates of 86/33/2 and one of the two replicates of 86/2SC/8, but was reduced in comparison with controls (Table 6.11).

Table 6.11 Sporulation of isolates in the presence of prochloraz as compare with controls

Isolate	Rep.	DWA	2PRO DWA
86/2SC/11	1	+++	-
	2	+++	-
86/2SC/8	1	+++	+
	2	+++	-
86/33/2	1	+++	+
	2	+++	+
86/86/18	1	+++	-
	2	+++	-

+++ = prolific sporulation
++ = some sporulation
+ = a little sporulation
- = no sporulation

Table 6.12 Sporulation of 3 isolates on DWA and proportions of hyphal plugs sporulating in the presence of prochloraz

Isolates	86/36/9	86/86/6	86/78/18
Sporulation on DWA rep. a)	+++	+++	++++
rep. b)	+++	+++	++++
rep. c)	+++	+++	++++
On 2PRO DWA			
no. plugs producing:-			
+++ growth	12	2	38
++	19	34	0
+	5	3	1
	4	0	1 *
	40	39	40

++++ = very prolific sporulation
+++ = prolific sporulation
++ = some sporulation
+ = little sporulation
- = no sporulation

* = pseudoparenchyma (description after Deacon, 1973)

The majority of hyphal plugs from each isolate produced spores on prochloraz, although mostly in reduced numbers. Isolate 86/78/18, of SF morphology, sporulated prolifically on control plates and 38 of the 40 treatment plates sporulated similarly . Not all conidia

produced on PRO DWA were capable of germination on prochloraz. Isolates 86/36/6y* and 86/86/18d* exhibited the same variation in growth on prochloraz as did the previously tested mass-mycelial isolates.

6.4.3.7. Stability of prochloraz insensitivity in *Pseudocercosporella*

1. Of the 54 plugs transferred to 1PRO PDA from colonies growing on PDA, 38 grew.

2. All 20 plugs not growing on 1PRO PDA, resumed growth when transferred to PDA.

3. Of the 51 plugs transferred from PDA to 2PRO PDA, 17 grew, of which 4 grew at a fast rate, 10 at a slow rate and 3 at a very slow rate.

6.4.3.8. Stability of prochloraz sensitivity in *Pseudocercosporella*

Of the 26 plugs (sensitive on 1PRO PDA) which resumed growth when transferred to PDA, 24 grew after a second transfer to 1PRO PDA. Of these, 6 grew at a slower rate to the other 18.

* originating from a single conidium

6.4.4 Discussion

2PRO PDA was used for testing responses of *Pseudocercosporella*, as isolates of *R. secalis* known to be prochloraz-sensitive, were completely inhibited by this media. Some growth on prochloraz-amended media was recorded for all tested N.Z. isolates of *Pseudocercosporella*, however some isolates had very few hyphae able to respond in this way and were only detected when many plugs from a colony were tested. Growth was often slow, but by keeping plates for up to four weeks, growth was detected. It is likely that isolates tested in the original 1983/84 survey would have exhibited a range of sensitivities if the tests had have been replicated in larger numbers.

Growth rate varied between hyphal plugs and usually originated from small points of the plug (Figs. 6.2 and 6.3) rather than from the whole plug as is the case with benomyl resistance. Growth of *Pseudocercosporella* occurred on all tested concentrations of prochloraz, ranging from 0.1 - 200 ug a.i. ml^{-1}, with the majority growing at 0.1 and 2.0 ug a.i. ml^{-1}. The proportion of hyphal plugs producing growth on prochloraz was consistent within an isolate, but varied between isolates. The insensitive response whereby only a proportion of hyphal plugs from isolates, originating from either mycelium or a single conidium, grew on prochloraz, was repeatable through generations.

The initial sporulation test on prochloraz-amended agar showed sporulation to occur in some isolate replicates but not others, which was similar to the variation found in mycelial tests. The second sporulation test, employing a large number of replicates, indicated that only some hyphae were sporulating, rather than all hyphae sporulating at a reduced rate.

Fungicide resistance has been defined by Delp and Dekker (1985) as the '..stable, inheritable adjustment by a fungus to a fungicide, resulting in a less than normal sensitivity to that fungicide (toxicant).' To be heritable, a response must be under direct genetic control. The inheritance of nuclear genes is easy to demonstrate in haploid fungal isolates. If there are genes present in

Pseudocercosporella which are controlling the prochloraz insensitivity, they appear to be replicating at a rate different to that of cell division which suggests they could be either under some form of regulation or be dependent upon gene amplification, of which there are examples with cultured plant cells (Donn *et al.*, 1984). It is possible that the genes are located extrachromosomally, as they would then not be subjected to the recombination and segregation that sexually reproduced genes are. Genes on mitochondria (Diacumakos *et al.*, 1965), plasmids and viruses (Fincham et al., 1979) and dsRNA (Van Alfen *et al.*, 1975) have been demonstrated to play a role in the metabolism of other fungi. Segregation ratios showed dodine-resistance in *V. inaequalis* to be under the control of two genes but, a poor fit of one cross to the ratios suggested additional factors to be operating (Polach, 1963).

Prochloraz insensitivity at different levels has been found in isolates obtained from an unsprayed population. It is unlikely that in the absence of a selection pressure, genes would have accumulated from a few rare mutations. It would be more likely that the genes function in the fungus, and are detected in the presence of prochloraz. Although an individual gene remains inherently stable, an insensitive response need not be a permanent and stable feature of an isolate. It is interesting that isolate G64344 (*P. aestiva*) was completely resistant to prochloraz. This may have been a rare mutant or the resistance could be a feature of *P. aestiva* isolates.

In the study of stability of insensitivity, 38 of the 54 plugs originally insensitive retained their insensitivity. This proportion was greater than the original insensitive numbers (25/54), suggesting some selection for insensitivity genes may have occurred on the first prochloraz plate. The response was similar at the higher rate of prochloraz, however fewer plugs grew. All plugs that had not grown on prochloraz-amended agar, grew following transfer back to PDA, demonstrating that although not capable of exhibiting an insensitive response they had remained alive during their exposure to the prochloraz-amended agar.

If spontaneous or induced mutation to insensitivity occurred in isolates during the period initially spent on prochloraz-amended media

when there was no growth, then the mutation rate was extremely high. Growth should therefore have been detected on the prochloraz-amended agar, and larger proportions of plugs would have grown in earlier tests, particularly after the plates had been kept for a few months. Assuming mutation had not occurred, genes for insensitivity must have been present during the initial screening on prochloraz.

Responses, similar to those found in isolates on prochloraz-amended medium, were found in isolates on media amended with DPX H6573, hence the effect is not unique to fungicides of either the imidazole or triazole group, but to the broader range of DMIs. It may be that some hyphae have sufficient ergosterol to continue growth for a limited period in the presence of these ergosterol-biosynthesis inhibitors. The responses observed, however, suggest an initial lag period occurs for some isolates before growth becomes established. The hyphae could be capable of producing ergosterol at a reduced rate, and if it builds up in certain regions, growth becomes possible. The question, as to why this occurs only in some hyphal plugs and not others, and the reason for the variation between isolates remains unanswered.

Pseudocercosporella isolates responded similarly on plates containing mixtures of fungicides as expected from responses to individual treatments. The two fungicides retained their individual effects on the isolates and there was no interaction.

In the field, prochloraz is not as effective as benomyl in the control of *Pseudocercosporella* in the absence of benomyl-resistant isolates. Even so, the results *in vitro* need not reflect responses of the pathogen exposed to the fungicide when growing on its host plants. The responses *in vitro* are at concentrations around the level of commercial application, although the radial mycelial growth EC50 values are lower. Unfortunately, the chemicals are phytotoxic at rates only slightly higher.

7.0 FIELD SPRAYING TRIAL AND STRATEGIES FOR CONTROL OF EYESPOT

7.1 INTRODUCTION

7.1.1 Effective disease control

Eyespot is slow to develop during the season, so by controlling early season infection, disease levels can be kept low. Current recommendations for its control (Witchalls and Close, 1971) cite a single fungicide application at G.S. 31. Factors relating to the application of chemicals are as important as chemical efficacy. The addition of an adjuvant may be required to prevent a chemical being washed away by rain before being taken up by a plant. In the case of eyespot control, however, light rain could actually improve coverage of target sites, assuming the chemical does not run off the plant. If spraying is left until late in the season, plants will have become dense and penetration of the crop canopy difficult. Adequate coverage is important not only for disease control but also for the prevention of a build-up of fungal isolates resistant to the chemical.

7.1.2 Field spraying trials for eyespot control

A long-term field experiment in Germany, monitoring effects of benomyl on the development of resistance in populations of *Pseudocercosporella*, demonstrated a steady, but slow, increase in resistance in the first five years (Fehrmann *et al.*, 1982). After this time, however, a dramatic increase in resistance occurred in sprayed plots, with unsprayed plots still containing only low-level resistance (Fehrmann, 1984). This suggests that resistance could be increasing exponentially with a threshold level required before control failure occurs.

Other trials have investigated effects of both benomyl and prochloraz on the different *Pseudocercosporella* pathotypes (Bateman *et al.*, 1986; Hoare *et al.*, 1986). In one of these (Bateman *et al.*, 1986) numbers of benomyl-resistant isolates increased in the presence of benomyl treatment but declined in its absence. An increase in numbers of SF strains relative to FE strains occurred across the trial but was

considerably increased in prochloraz-treated plots. The sample size of isolates from these plots, however, was small. Spray drift was not considered to explain increases in proportions of benomyl resistance in plots not treated with benomyl as plots were separated by 12m buffers. The possibility of the fungus spreading between plots was not considered, but from experimental work carried out during this study, it would be expected that spread would certainly have been possible over this distance. Increases in proportions of SF isolates in prochloraz-treated plots have been described elsewhere, but these were generally benomyl-sensitive (Hoare *et al.*, 1986). Although a strong association between benomyl resistance and SF morphology has been noted (Griffin and Yarham, 1983; Hollins *et al.*, 1985; King and Griffin, 1985), applications of prochloraz did not appear to affect benomyl sensitivity. All plots sprayed with benomyl or prochloraz, in contrast to unsprayed plots, appeared to select for SF isolates (Bateman *et al.*, 1986; Hoare *et al.*, 1986). The association between benomyl resistance and SF morphology could perhaps be expected only to be strong at sites sprayed with benomyl.

The present dominance of SF strains in U.K. eyespot populations could be explained by the suggestions that optimum environmental conditions for infection may differ between SF and FE types and that the two types may also differ in their interactions with other pathogens and secondary colonisers of eyespot lesions (Bateman *et al.*, 1986). The fungicides may also be having an effect on the secondary colonisers which in turn will affect the relationship between the colonisers and the different *Pseudocercosporella* pathotypes.

Development of benomyl resistance has been delayed by applying mixtures of carbendazim and prochloraz. Two sprays of prochloraz, each a half-rate, were found in one of three seasons to be significantly more effective than a single spray at the full rate. It was suggested that the optimum application time for prochloraz was not always the recommended spray at G.S. 31 (Hoare *et al.*, 1986).

7.1.3 Aim of field trial

To assess relative effects of benomyl and prochloraz application on both disease control and the build-up of fungicide resistance in low and high-density plantings. The incorporation of Citowett as an adjuvant with the fungicides was also studied.

7.2 MATERIALS AND METHODS

7.2.1 Plants and inoculum

A 44 X 5m site was sown with wheat (cultivar Rongotea) in Southland on 18/9/86. A Wintersteiger seedmatic drill was used to sow 1 X 1.25m plots to give seeding rates of 2.5g (≒65 grain) and 5 g (≒125 grain) per metre row. Cereals had been sown at the site in the previous season, although the level of eyespot was unknown. Ground preparation was kept to a minimum with just grubbing and rolling, in the hope that exposed stubble would provide inoculum. Artificial inoculum was also added to ensure high disease levels.

All plots were inoculated on 22/10/86 when plants were at G.S. 12. Inoculum consisted of FE and SF isolates but isolates were undescribed in terms of benomyl-sensitivity. Inoculum was spread evenly between the rows of plants at the rate of 15g m^{-2}.

7.2.2 Experimental design

A split-plot design was used with chemical sprays being the main treatment and plant density the sub-treatment. Land limitations precluded the use of buffers within the trial. Oat buffers (cultivar Ohau) one plot in width, were sown around the trial to eliminate edge effects. The field plan appears in Fig. 7.1 and the trial itself in Fig. 7.2. There were four replications, with treatments and sub-treatments randomised within each.

Fig. 7.1 Field plan of fungicide spray trial with replicate one in detail

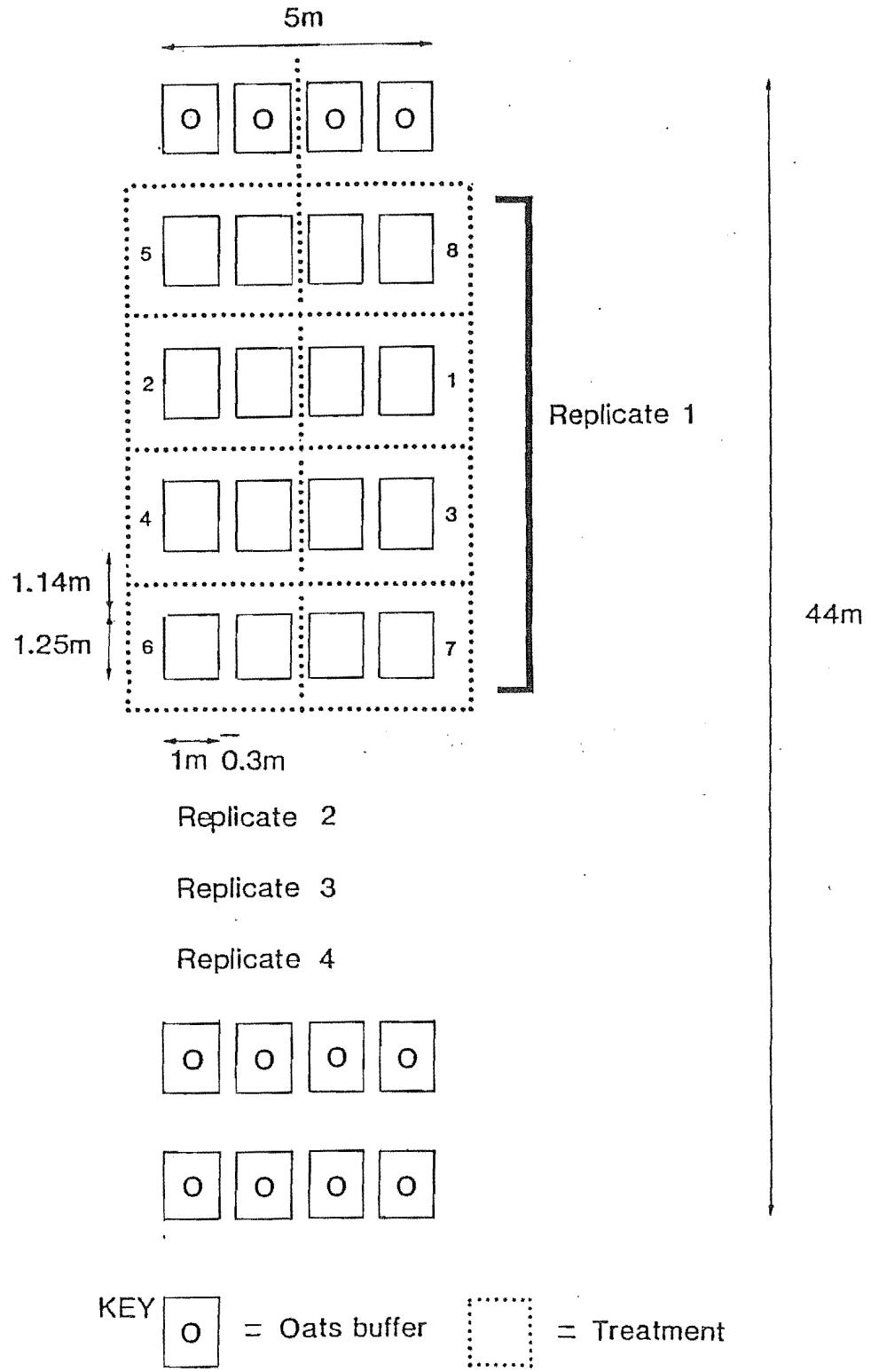


Fig. 7.2 Site of field spraying trial with oats buffer in foreground and treatment plots of Rongotea behind.

7.2.3 Spraying

Treatment plots were sprayed to run-off on 22/11/86, using a hand-operated knapsack sprayer.

Treatments

1. benomyl (500g a.i. $h\bar{a}^1$ or 0.625g a.i./1.25m^2 plot)
2. benomyl (500g a.i. $h\bar{a}^1$) + Citowett (0.025%)
3. prochloraz (450g a.i. $h\bar{a}^1$)
4. prochloraz (450g a.i. $h\bar{a}^1$) + Citowett (0.025%)
5. Tank mix of benomyl (500g a.i. $h\bar{o}^1$) + prochloraz (450g a.i. $h\bar{a}^1$)
6. Tank mix of benomyl (500g a.i. $h\bar{a}^1$) + prochloraz (450g a.i. $h\bar{a}^1$) + 0.025% Citowett
7. Tank mix of benomyl (250g a.i. $h\bar{a}^1$) + prochloraz (225g a.i. $h\bar{a}^1$)
8. Unsprayed control

Citowett was used as the adjuvant.

7.2.4 Plant analyses

Plant establishment was recorded on 9-10/12/86, by counting plants in a metre length of the third row in each plot. Plants were scored for disease severity early in the season on 10-12/12/86 at G.S.30-31. In this way, if minimal disease symptoms were present later in the season it would be known if they were a result of late infection (when chemical persistence may have decreased) or interrupted lesion development occurring early in the season in response to chemical sprays. Plants were again scored on 7/1/87 when at G.S. 65.

7.2.5 Scoring

As early season plants had to be scored non-destructively, a scale of 0-5 was devised for assessing symptoms where:

0 = no infection
1 = slight lesion formation
2 = complete lesions
3 = numerous severe lesions
4 = plant tissue collapse at sites of lesions
5 = lodging

A score was given to each plot based on average plant scores.

This scale was used later in the season, however, individual tiller scores were noted. A minimum sample of 20 tillers per plot was taken.

Isolates from severe lesions were described in terms of colony morphology and evaluated *in vitro* for sensitivity to benomyl.

7.2.6 Statistical analysis

The following disease severity index (an extension of that reported by Scott and Hollins, 1974) was used to standardise disease scores per plot:

$$\frac{y = a(0) + b(1) + c(2) + d(3) + e(4) + f(5)}{\text{total no. tillers}} \times \frac{100}{5}$$

where a-f = no. of tillers in each category.
(0-5) = score categories

Analyses of variance and covariance were determined using a GENSTAT computer package. Appropriate data and directive files were created (Appendix 7).

Randomisations

Treatment randomisations for plots 1-64

REPLICATE 1

5	(61 A	62 B)		8	(63 B	64 A)	
2	(57 B	58 A)		1	(59 A	60 B)	
4	(53 A	54 B)		3	(55 B	56 A)	
6	(49 B	50 A)		7	(51 A	52 B)	

REPLICATE 2

6	(45 A	46 B)		7	(47 A	48 B)	
8	(41 B	42 A)		5	(43 B	44 A)	
3	(37 B	38 A)		2	(39 A	40 B)	
1	(33 A	34 B)		4	(35 A	36 B)	

REPLICATE 3

2	(29 B	30 A)		5	(31 A	32 B)	
3	(25 B	26 A)		8	(27 A	28 B)	
4	(21 B	22 A)		6	(23 A	24 B)	
7	(17 B	18 A)		1	(19 B	20 A)	

REPLICATE 4

1	(13 B	14 A)		8	(15 A	16 B)	
7	(9 A	10 B)		2	(11 A	12 B)	
4	(5 A	6 B)		3	(7 B	8 A)	
5	(1 A	2 B)		6	(3 A	4 B)	

Treatments

A = low-density plants
B = high-density plants

1 = benomyl
2 = benomyl + Citowett
3 = prochloraz
4 = prochloraz + Citowett
5 = benomyl/prochloraz
6 = benomyl/prochloraz + Citowett
7 = 0.5 rates benomyl/ 0.5 rates prochloraz
8 = control

7.3 RESULTS

Plant establishment was affected by bird feeding and irregular soil texture as a result of the limited preparation before sowing. Establishment varied significantly between plots and did not coincide with intended densities. Variation in disease severity in response to chemical control could therefore not be studied with respect to plant density. Disease scores were adjusted with covariates to remove variation resulting from uneven plant densities, allowing comparisons of treatment data.

Early season disease scores (Table 7.1) show slightly higher levels of disease in plots unsprayed or treated with benomyl compared with other treatments.

Table 7.2 lists scores for plant density and disease severity obtained later in the season. Statistical analyses (Table 7.3) show disease scores obtained between treatments to be highly significant ($P<0.001$). Mean disease severity index in the absence of chemical treatment was 55.48. There was no lodging in the trial. Benomyl sprays reduced disease to 32.79 and the addition of Citowett increased efficacy of benomyl sprays giving a disease index of 15.99.

Prochloraz treatment reduced disease to 11.26 with the addition of Citowett having no significant effect. Treatment with full rates of both benomyl and prochloraz gave the best control with a disease severity index of only 4.50. The addition of Citowett again had no effect. The tank-mix of half rates of each of benomyl and prochloraz provided control equivalent to that of full rates of prochloraz alone.

In vitro benomyl resistance and colony morphology of isolates obtained from the trial are given in Table 7.4. Isolates were only obtained from four of the eight control plots and one of the benomyl-resistant isolates was obtained from a corner block. Ten of the 15 isolates (66%) collected from control plots were SF as were the two benomyl-resistant isolates. Twenty one of 22 isolates (95%) obtained from

seven plots treated with full rates of benomyl, were benomyl-resistant. A total of 17 isolates from these plots were FE and all were benomyl-resistant. The other five isolates were SF, of which four were benomyl-resistant. Of the two isolates collected from plots treated with half rates of each of benomyl and prochloraz, one was FE and benomyl-resistant and the other was SF and benomyl-sensitive. The one isolate obtained from the plots treated with full rates of prochloraz was FE and benomyl-resistant.

TABLE 7.1

Chemical spraying trial disease scores (10-12/12/86)

Treatment	Replicate 1		2		3		4	
Density	full	half	full	half	full	half	full	half
1.	1	1	2-3 (4)	2-3	0-1	0-1	2(4)	1(4)
2.	0(1)	0-1	2-3	1(3)	3	2	3-4	0(1)
3.	0-1	1(2)	1	1	1-2	2-3	0(1)	1
4.	1	0-1	3	0-1(2)	0(1)	0-1	0(1)	0(3)
5.	0-1	1	1(2)	1	0(1)	1-2	1-2	1-2
6.	0-1	0-1	2-3	0(1)	1-2	0-1	1-3	0-1
7.	1-2	1	2(3)	1-2(3)	1	1	0(1)	3
8.	1	1-2	2	2-3(4)	3	2	3	2

Scores represent average disease in each plot with scores in brackets describing maxima

Table 7.2 Chemical spraying trial final disease scores

FINAL SCORES

PLOT	TRTMT	SOWING RATE	PLANT DENS.	SCORES (frequency) 0	1	2	3	4	5	DISEASE SEVERITY INDEX
1*	5	1	12	17	2	0	0	0	0	2.10
2*	5	2	16	16	1	0	0	0	0	1.77
3	6	1	13	23	0	0	0	0	0	0.00
4	6	2	12	21	3	0	1	2	0	11.20
5	4	1	9	18	15	2	0	0	0	10.86
6	4	2	10	20	7	0	0	0	0	5.19
7	3	2	12	15	12	1	0	0	0	10.00
8	3	1	18	28	0	0	0	0	0	0.00
9	7	1	4	16	4	3	2	0	0	12.80
10	7	2	8	9	7	3	8	0	0	27.41
11	2	1	5	23	7	0	0	0	0	4.67
12	2	2	12	6	6	4	8	3	0	37.04
13	1	2	15	2	1	8	5	8	4	60.00
14	1	1	12	6	15	4	1	4	0	28.00
15	8	1	3	0	0	0	18	4	0	63.64
16	8	2	15	0	4	3	7	14	0	62.14
17	7	2	19	18	8	0	0	0	0	6.15
18	7	1	10	12	8	2	0	0	0	10.91
19	1	2	12	6	14	4	5	0	0	25.52
20	1	1	11	2	9	4	6	0	0	33.33
21	4	2	4	19	5	8	1	1	0	16.47
22	4	1	10	12	9	4	0	1	0	16.15
23	6	1	8	12	9	1	1	0	0	12.17
24	6	2	13	29	0	0	4	0	0	7.27
25	3	2	9	19	2	2	1	0	0	7.50
26	3	1	9	14	8	0	5	0	0	17.04
27	8	1	7	0	4	3	12	10	0	59.31
28	8	2	14	0	1	6	5	5	7	69.16
29	2	2	8	27	9	2	0	1	3	15.24
30	2	1	13	18	4	3	3	3	0	20.00
31	5	1	10	30	4	0	0	0	0	2.35
32	5	2	14	25	6	0	0	0	0	3.87
33	1	1	12	12	7	1	3	3	0	23.08
34	1	2	10	5	12	8	5	2	0	31.88
35	4	1	4	13	4	4	3	0	0	17.50
36	4	2	15	15	4	4	1	3	0	20.00
37	3	2	9	20	12	3	0	0	0	8.00
38	3	1	16	8	7	7	0	0	0	19.09
39	2	1	0	11	9	3	5	1	0	23.45
40	2	2	13	7	8	7	7	4	0	35.76
41	8	2	19	0	3	7	7	5	4	60.00
42	8	1	7	0	10	5	10	3	0	44.29
43	5	2	3	19	2	3	0	0	0	6.66
44	5	1	17	24	2	0	0	2	0	7.14
45	6	1	5	25	0	0	0	0	0	0.00
46	6	2	14	26	2	0	0	0	0	1.43
47	7	1	6	14	9	1	0	0	0	9.16
48	7	2	15	14	6	1	1	1	0	13.04

49*	6	2	9	15	1	1	0	0	0	3.53
50	6	1	10	20	7	1	0	0	0	6.43
51	7	1	5	14	9	1	0	0	0	9.16
52	7	2	16	20	3	2	2	0	0	9.63
53	4	1	13	35	1	0	0	0	0	0.56
54	4	2	13	28	6	0	0	0	0	3.53
55	3	2	6	18	7	2	1	0	0	10.00
56	3	1	12	28	2	1	1	0	1	7.27
57	2	2	7	16	10	2	1	0	0	11.72
58	2	1	8	17	5	2	0	0	0	7.83
59	1	1	12	13	6	6	2	0	0	17.78
60	1	2	11	16	3	2	4	2	3	28.00
61	5	1	8	26	1	1	0	0	0	2.14
62	5	2	10	29	2	0	1	0	0	3.13
63	8	2	17	6	5	1	9	4	4	48.28
64	8	1	10	4	15	4	3	1	0	26.67

TRTMT = Treatment
Dens. = Density

* some missing values (tiller scores) from these plots

Table 7.3 Analysis of variance and means of treatment scores from chemical spraying trial

ANOVA - adjusted for covariate (actual plant density)

Variate : Disease score

Source of variation	DF	MS	F PR
Rep. Stratum			
Covariate	1	162.53	0.554
Residual	2	327.71	
Total	3	272.65	
Rep.Treatment.Stratum			
Treatment	7	2349.01	<0.001
Covariate	1	526.86	0.008
Residual	20	61.53	
Total	28	650.02	
Rep.Treatment.Density.Stratum			
Covariate	1	265.00	0.038
Residual	31	56.63	
Total	32	63.14	
Grand Total	63		

Total number of observations = 64

TABLE OF MEANS (adjusted for covariate and actual plant density)

Grand mean 18.37

Treatment (means adjusted for actual plant density)

1. benomyl	32.79	
2. benomyl and Citowett	15.99	
3. prochloraz	11.26	
4. prochloraz and Citowett	10.03	
5. benomyl/prochloraz	4.50	
6. benomyl/prochloraz and Citowett	4.99	
7. 0.5 ben./0.5 pro.	11.94	
8. control	55.48	SED = 4.009

Table 7.4 Isolates from chemical spraying trial

ISOLATE **		GROWTH TYPE	2BEN.*	TREATMENT
F13	1	FE	Res.	1
	2	SF	Res.	1
	3	FE	Res.	1
	5	FE	Res.	1
	6	FE	Res.	1
	20	FE	Res.	1
F16	1	SF	Sens.	1
F19	20	FE	Res.	1
F33	1	FE	Res.	1
	100	SF	Res.	1
	200	FE	Res.	1
F59	2	SF	Res.	1
	100	FE	Res.	1
	200	FE	Res.	1
	300	SF	Res.	1
F12	1	FE	Res.	2
	2	FE	Res.	2
	3	FE	Res.	2
F39	1	FE	Res.	2
	2	FE	Res.	2
	66	FE	Res.	2
F57	1	FE	Res.	2
F20	2	FE	Res.	4
F15	0	FE	Res.	7
	1	SF	Sens.	7
F14	1	FE	Res.	8
F27	30	FE	Sens.	8
	33	SF	Sens.	8
F63	1	FE	Sens.	8
	2	SF	Sens.	8
	5	FE	Sens.	8
	6	FE	Res.	8
	7	FE	Sens.	8
F64	1	FE	Sens.	8
	2	SF	Sens.	8
	3	SF	Sens.	8
	4	SF	Sens.	8
	7	FE	Sens.	8
	10	FE	Sens.	8
	100	FE	Sens.	8

* isolate resistant (Res.) or sensitive (Sens.) to 2ug a.i. ml^{-1} benomyl (2BEN.) *in vitro*

** F13 1 = isolate 1 from fungicide trial plot 13.

Treatments:-

1.	benomyl	14/15 Res.	5 reps
2.	benomyl + Citowett	7/7 Res.	1 rep.
4.	prochloraz + Citowett	1/1 Res.	1 rep.
7.	0.5 ben./0.5 pro.	1/2 Res.	1 rep.
8.	control	2/15 Res.	4 reps

7.4 DISCUSSION

7.4.1. Field trial

It was unfortunate that effects of plant density on the success of fungicide application could not be determined, however the trial clearly demonstrated the rapidity with which benomyl-resistant isolates build up in the presence of benomyl.

As control plots scored a disease severity index of only 55.48, it was fortunate that the trial was artificially inoculated. The natural inoculum level was obviously very low. Benomyl sensitivity of the isolates was unknown, however as 2 of the 15 isolates collected from control plots were resistant, this may be considered to be the initial proportion across the trial.

The isolate sample size was too small to make inferences on the effects of the treatments on morphological types, however it appears that benomyl had no effect in increasing the number of SF isolates. Benomyl provided the least control of over-all disease and the figures for benomyl resistance suggest that resistant isolates were selected and then proliferated in benomyl sprayed plots.

Control by prochloraz was markedly better than that by benomyl and the mean disease scores obtained describe numerous tillers of very low-level infection rather than occasional highly diseased or 'escape' tillers. As infection was minor, only on the outer leaf sheaths, it was difficult to successfully isolate from them, hence only one isolate was obtained from these plots. Early season disease scores indicated the presence of low-level infection. Prochloraz spray application was efficient with low late-season disease scores reflecting chemical efficacy. Disease development of young lesions had obviously been inhibited by prochloraz so numbers of infections were high but later development would have been inhibited.

Prochloraz has been reported to provide poorer control than benomyl of *Pseudocercosporella* when disease is severe (Griffiths, 1983) as benomyl-sensitive isolates would have been prevalent. That prochloraz had a marked effect in reducing disease levels, particularly when

mixed with benomyl is consistent with results of Barnes *et al.*, (1983) and Matthews *et al.*, (1985).

Spraying to run-off was made possible with the use of a backpack sprayer. The coverage from this is likely to be better than that obtained with hydraulic booms commonly used commercially. If controlled droplet application is used commercially, however, the incorporation of low spray volumes and small droplet sizes should mean higher concentrations of active ingredient reach target sites (Bayer Australia Ltd, 1976). It is likely that Citowett increased the retention of benomyl, increasing disease control, but had no effect on the retention of prochloraz. An emulsifiable concentrate of prochloraz was used in this trial, hence better coverage and retention could be expected than that with the wettable powder formulation of benomyl. Citowett also had no effect on retention of the mixture of benomyl and prochloraz. The emulsifier in prochloraz must also have aided the retention of benomyl.

7.4.2. Spray application strategies for preventing a build-up of fungicide resistance

Recommendations for disease control must be considered not only from direct field data describing the performance of chemicals, but also for the best use of these chemicals to ensure their continued success for as long a period as possible. The technique of chemical application to target crops is important for the prevention of problems with fungicide resistance. The three factors discussed below play a major role in the success of chemical application.

1) Fungicide application time

The larger the pathogen population existing at the time of spraying and assuming a constant mutation rate, the higher will be the number of mutations for insensitivity. Numbers of *Pseudocercosporella* spores reaching a crop as primary inoculum and successfully establishing infection, would be greatly outnumbered by secondary spores. Screening pressures exerted on a secondary pathogen population by a curative

spray will far exceed those of preventative sprays applied to control primary inoculum. When high inoculum pressures are expected, chemicals should be sprayed preventatively before disease symptoms appear in a crop when a target population is still small. When pressures are likely to remain low it would be preferable not to spray at all.

Forecasting disease pressure can best be based on the recognition of disease levels in a previous crop at the same site, the growing of highly susceptible cultivars, particularly those of wheat, the time of sowing and weather conditions during the post-emergence period.

2) Coverage

Complete crop coverage, as is required for protectant fungicides, can cause the death of a sensitive population component, resulting in a swing to total population resistance. This occurs particularly with pathogens in the flag leaf, such as Septoria tritici blotch (Fisher and Griffin, 1984) where coverage is likely to be 100%. In such situations, control with benomyl is directly proportional to the percentage of benomyl-sensitive fungal strains present within the population. In contrast, control of eyespot which is a disease at the base of the wheat plant relies on the benomyl fungicide being sprayed to the base of the plant and being translocated upwards. Benomyl-sensitive isolates of *Pseudocercosporella* were frequently recovered from fields sprayed with benomyl (Chapter 6) indicating a failure in chemical distribution with the isolates being 'escapes'.

Fig. 7.3 Effect of different fungicide rates on the survival of a fungal population of mixed insensitivities

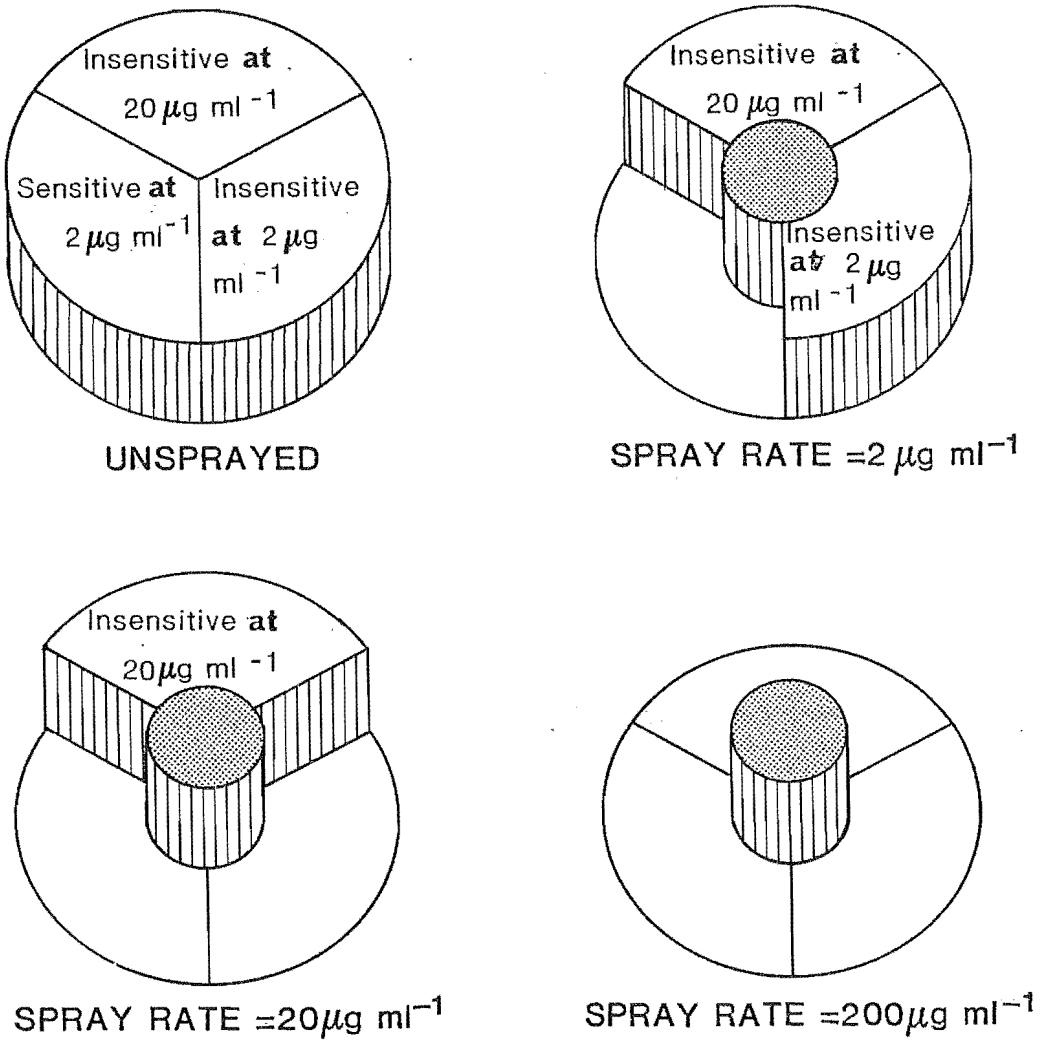


Fig. 7.3 represents a hypothetical fungal population containing three levels of fungicide sensitivity. This is very simplistic as it is unlikely that any population would have three such specific and equally proportioned levels. The effects of different spray rates are limited by coverage which in this model is set at 90%. Even so, 10% failure will aid a rapid build-up of the insensitive component of a population.

3) Fungicide mixes

Simulation models (Kable and Jeffery, 1980; Josepovits and Dobrovolszky, 1985) have shown that when spray coverage is incomplete, resistance build-up is delayed by mixing fungicides of different modes of action.

Fig. 7.4 depicts selection pressures acting on a hypothetical population, in which unlinked insensitivity to two fungicides is present.

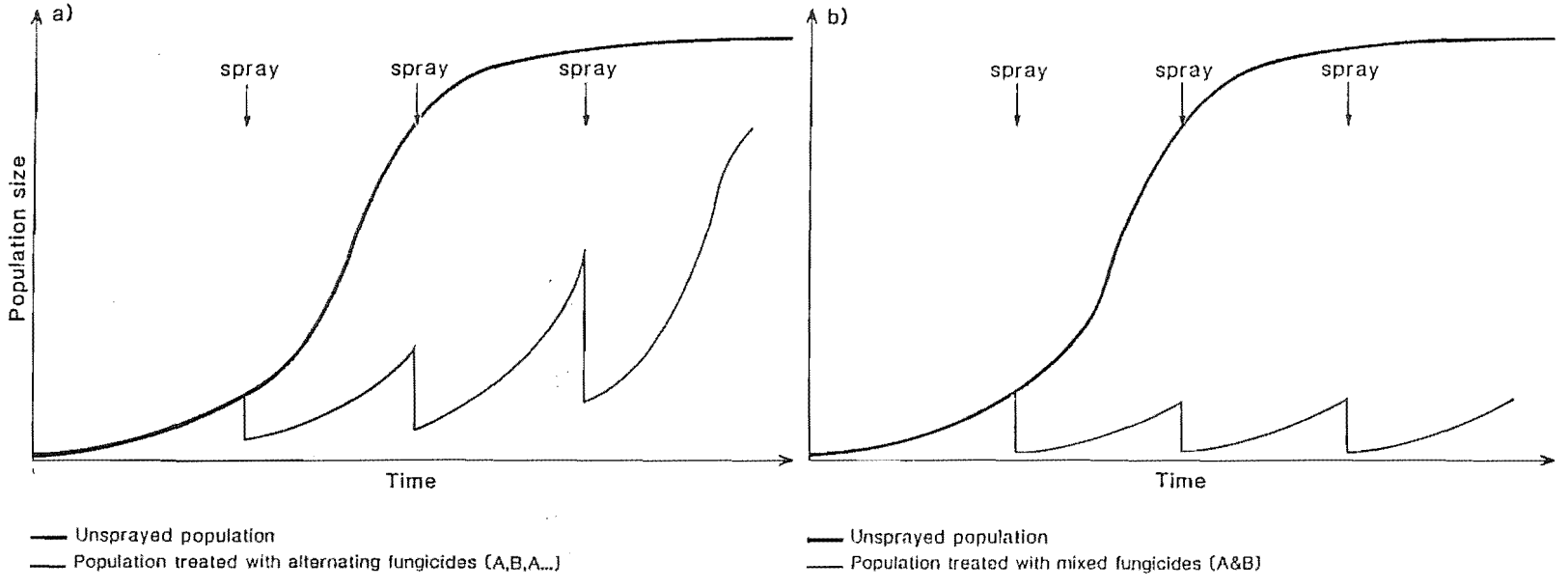
Under application of either fungicide, :-

- Control of isolates sensitive to both fungicides will be proportional to spray coverage.
- Isolates sensitive to only one fungicide, will be screened on exposure to that fungicide, and conversely selected on exposure to the other.
- Isolates with insensitivity to both fungicides will increase following exposure to either fungicide. Such isolates will be present at very low levels.

The surviving 'escape' population makes alternation of different chemical groups only a temporary measure in delaying a build-up of insensitivity. Each respective spray allows for a base-line increase in numbers of alternate isolate types. Fungicide mixtures maintain a constant pressure on isolates of either insensitivity. When mixtures are employed, concentrations of chemicals may not be as critical as resistant isolates should be controlled by the alternative chemical.

The promotion of fungicide mixtures should not be merely for chemicals of differing modes of action, but for chemicals which promote differing pathogen responses.

Fig. 7.4 Effect of a) alternating and b) mixed fungicide regimes on the survival of two fungal population components



7.4.3 Chemical strategy for eyespot control

In light of the importance of employing mixtures of fungicides, as indicated above for the prevention of resistance problems, the results of the chemical spray trial point to the possibility of developing an improved strategy for eyespot control. It may be of long-term value to control eyespot with a mixture of benomyl and prochloraz, with each component at half rates. When taking cost into consideration, such a strategy would be more economical for the grower than would be the use of full rates, as the cost of prochloraz is significantly higher than that of benomyl.

Provision must also be made for the control of other diseases. This strategy may prove useful in the control of Septoria tritici blotch and may prevent fungicide resistance problems developing for this disease also. Control of scald of barley is currently undertaken by spraying with prochloraz. With controlled droplet application, enough active ingredient may still reach target sites from half rate sprays, for control to be maintained.

The results from the trial taken in view of the above considerations, suggest that the application of mixtures of benomyl and prochloraz, with half rates of each component, would be a strategy for improved eyespot control in Southland.

EXPERIMENTAL WORK

PART 3 - Eyespot disease in the field - epidemiology and host responses

8.0 HOST RESISTANCE/SUSCEPTIBILITY

8.1 INTRODUCTION

8.1.1 Origins of *Pseudocercosporella* and its wild hosts

Pseudocercosporella appears to have originated in the northwest of the U.S.A.. Sprague (1934) reported its occurrence on prairies originally bearing a *Festuca* sod-grass consociation. Indicator plants were described as *Festuca idahoensis* Elmer, *Agropyron* spp, *Balsamorhiza* spp, *Delphinium menziesii* DC, *Lomatium triternatum* (Pursh) Coult. and Rose and *Lithospermum ruderale* Dougl. ex Lehm. Pugill. At that time, exceptions to these associations were so few that Sprague suggested the disease would remain a relatively localised problem. The disease was not widespread before 1910, and locations where it did occur were very moist. Evidence suggests the fungus had existed for a long time on native grass in semi-arid conditions, but adapted to the more humid conditions of wheat crops. Numerous pasture grasses susceptible to *Pseudocercosporella* were described, although if sown sparsely most susceptible plants remained uninfected (Sprague, 1936). Many of the field-grown cereal cultivars considered resistant were actually 'escape' plants, with climatological factors or maturation time preventing infection. Sprague and Fellows (1934) stated that 'the relative susceptibility of varieties varies somewhat from year to year'. Even oats, *Triticum monococcum* L. and Oregon winter rye, which were considered to have some degree of resistance, became heavily infected when sufficient inoculum was added to the soil. All inoculated species of *Agropyron* were found to be susceptible to *Pseudocercosporella*, and this was considered to be the most susceptible genus, with *A. spicatum* (Pursh.) Scribn. and Smith and *A. inerme* (Scribn. and Smith) Rydb perhaps being the native hosts of the fungus. Various species of *Aegilops* and *Triticum* showed great differences in resistance and susceptibility to *Pseudocercosporella* and the possibility of resistant cereals being bred from hybrids between wheat and certain related grasses was suggested. *Agrostis*

palustris Huds., *Cynosurus cristatus L.*, *Festuca rubra* L. and *Poa nemoralis* L. were considered to have some resistance to eyespot.

8.1.2 Breeding cultivars for eyespot resistance

The French wheat cultivar, Capelle-Desprez, exhibits durable resistance to *Pseudocercosporella* and this resistance has been introduced into many European cultivars. The major nuclear component of this resistance was shown to be carried on chromosome 7A (Law *et al.*, 1976), but no genes have yet been characterised. Doussinault and Dosba, (1977) considered there is little possibility of developing superior resistance from transgressive segregants arising from crosses with Capelle.

Initial findings by Sprague (1936) of high levels of resistance in *Aegilops ventricosa* Tausch led to the development of screening programmes in the hope of finding suitable lines for incorporation into wheat breeding programmes. A hexaploid wheat line, VPM1, with intermediate resistance to eyespot between the least susceptible wheats and *Ae. ventricosa*, was produced from the following crosses: *Ae. ventricosa* X *Triticum persicum* Navilov X *T. aestivum* L. cv. Marne (Maia, 1967; Doussinault and Dosba, 1977). Resistance was particularly noticeable at the seedling stage with successive penetration of leaf sheaths of VPM1 being much slower than that of other varieties. Mycelia between the leaf sheaths has a different appearance in resistant lines, being spotted and dark brown (m type), in contrast to large black colonies (v type) produced in susceptible lines (Jahier *et al.*, 1978; Doussinault *et al.*, 1983). The distinction between mycelia is sometimes not clearcut.

Monosomic analysis of 'Roazon' produced by a cross between VPM1 and the cultivar 'Moisson' found resistance to be associated with chromosome 7D. The resistance was found to be not fully dominant at either seedling or adult stages (Jahier *et al.*, 1978).

A single, major dominant gene for resistance, Pch-1, was successfully transferred from tetraploid *Ae. ventricosa* (genomes DvDvMvMv) to hexaploid bread wheat *Triticum aestivum* (AABBDD), using the tetraploid wheat *T. turgidum* L. (AABB) as a bridge species (Doussinault *et al.*,

1983). It is not yet known whether the gene was transferred from the Dv or Mv genomes, however the high frequency of transfer obtained was that expected of a Dv gene. Recombination between the Dv and Mv chromosomes, however, could have occurred before the transfer. Lines more resistant to *Pseudocercosporella* than the parent VPM1 have been produced. These have probably resulted from recombination between Dv chromosomes of *Ae. ventricosa* and D chromosomes of wheat. At least one of the Mv chromosomes is involved in the resistance. It is not yet known if Pch-1 is located on chromosome 7D. The cytoplasm of *Ae. ventricosa* also appears to promote expression of resistance (Dosba and Doussinault, 1977).

8.1.3 Pathogenic variation

Durability of resistance depends not only on the complexity of the resistance, but on the origin of the resistance and the ability of a pathogen to adapt. *Pseudocercosporella* has the wide host pathogenicity typical of many necrotrophic cereal parasites. Variation in pathogenicity is greater in relation to different host species than cultivars. The introduction into wheat of resistance genes from other wild species may be less durable than that of genes from within the species (Scott and Hollins, 1980).

The division of isolates into pathogenic groups, namely W and R-types (Lange-de la Camp, 1966; Scott *et al.*, 1975) was extended to include couch-type (C-type) on the finding of isolates strongly pathogenic to *Agropyron repens* (couchgrass or twitch) (Cunningham, 1965, 1968, 1971, 1981). C-type isolates were virulent on wheat and barley but not rye and *Ae. ventricosa*, distinguishing them from R-types. W-type isolates are avirulent on couchgrass. *Pseudocercosporella* isolates infecting couch-infested cereal crops were predominantly C-type. W-types were only occasionally isolated from couch. C-types would be more likely to survive between seasons on *A. repens* around the periphery of fields. The relative fitness of C-type isolates is unknown and they have not been isolated from couch-free cereal fields (Cunningham, 1971).

Pseudocercosporella has been reported to be virulent on oats (Oort, 1936; Bawden, 1950; McKay *et al.*, 1956; Cunningham, 1968,1971). Oort

(1936) reported a severe attack resulting in lodging and described the fungal hyphae as being visibly more profuse in the central cavities of stems of oats than wheat or barley, and suggested an attack to be related to prevailing weather conditions. Lange-de la Camp (1967) provided evidence that differing responses of species to infection is temperature dependent. On the further testing of isolates, some were found to be more pathogenic on oats than on *A. repens*, with *Ae. ventricosa* being highly resistant (Cunningham, 1971).

R-type isolates have been found to be highly pathogenic to *Aegilops squarrosa* L. (line vent. 11), whereas W-type isolates were much less pathogenic to this and other *A. squarrosa* lines (Scott *et al.*, 1976). None of the isolates were highly pathogenic to *Ae. ventricosa*, however with pathogenicity having been found in the genus *Aegilops*, there is a higher possibility of adaptation of *Pseudocercosporella* isolates to *Ae. ventricosa* than has before been considered. Infection of barley was shown to be more affected by environmental changes than by differences between isolates. Its performance was more variable than that of other hosts and it was often infected less than rye (Lange-de la Camp, 1967).

Inoculation tests measuring the susceptibility/resistance of winter barley varieties to eyespot showed varieties to differ only slightly (Anon.,1982).

Significant differences have been found in the infection produced by R-type isolates in U.K. triticale varieties and breeding lines, demonstrating selection for triticales resistant to R-types. Cultivars Salvo and Lasko were as severely affected as susceptible wheat varieties, however, Warren and Torrs were only infected to the same degree as VPM1 (Scott and Hollins, 1985).

More resistant varieties had shorter basal internodes, short to medium straw and thick haulm walls. Following infection by *Pseudocercosporella*, crude fibre production in the lower internodes was inhibited. Varieties with highest crude fibre content in the absence of infection also had the lowest degree of attack and the least stem break in the presence of infection (Mielke, 1970).

Adaptation of isolates to a particular host has not readily been acquired after numerous passages through a host (Scott *et al.*, 1975). Such adaptation would not, however, be expected unless a mutation was induced which improved the pathogenicity.

8.1.4 Inoculation techniques

Inoculation techniques employed for pathogenicity studies have varied. In field trials, both natural infection (Dickens, 1964) and various artificial inoculation methods have proved successful. Inoculated wheat straw (Lupton and Macer, 1955) and colonised oatgrains (Sprague, 1936) have been spread around field plots. Macer (1966) suggested that field trials should be used only for plants in the later stages of breeding programmes when large quantities of seed become available. Seed from earlier stages of breeding, such as the selection of F1 plants of backcross generations, would be more suited to testing in a glasshouse or growth room.

Numerous methods have been employed for infecting potted cereal plants with *Pseudocercosporella*. Sprague (1931, 1934, 1936, 1937) used oat and barley grain inoculated with the fungus as inoculum and spread this on the soil at the rate of 4-6 inoculated grains to each sown seed. Glynne *et al.*, (1945) placed pieces of mycelial cultures of the fungus from PDA at the base of each plant and successfully obtained infection.

Lange-de le Camp, (1959) found it more effective to place inoculum on top of rather than within soil. Better results were obtained in a glasshouse on vernalised wheat kept at 10°C rather than 15°C.

Pseudocercosporella has also been cultivated on moist cornmeal-sand medium which was distributed evenly over the soil surface of potted plants (Dickens, 1964).

Macer (1966) inoculated split-straw cylinders from internodal wheat straws and placed these over emerging coleoptiles. The level of resistance of seedlings to *Pseudocercosporella* was found to be inversely proportional to the depth of penetration of the leaf

sheaths, and was directly correlated to field development of the disease, as measured by lodging and yield loss. The technique was considered artificial as infection occurred at a very early stage of seedling development by direct hyphal penetration. The seedling is also attacked simultaneously at several points which is not necessarily what occurs in nature. A layer of sand was placed around the straw cylinders to increase support and prevent them from moving upwards with the elongating stems. A moist microclimate suitable for infection was also maintained, however infection develops unnaturally as it is in continual darkness. Evans and Rawlinson (1975) modified this method by inserting sterilised filter paper, impregnated with *Pseudocercosporella* conidia, into a glass tube and inverting this over a seedling, in place of a split-straw cylinder. This overcame problems inherent with the use of a natural and variable substrate whilst allowing infection by both mycelia and spores above soil level. Fungal mycelium was found to infect leaf sheaths more rapidly when kept at a 10°C night/15°C day temperature regime rather than at 5°C night/10°C day, however, the number of living, infected leaf sheaths was less in the higher temperature regime. Defosse (1967) reported the optimal temperature for infection of the first leaf sheath was at or below 15°C whilst that for progress to the second leaf sheath was nearer 20°C.

Differences between resistant and susceptible cultivars were found to be distinguished during very early stages of infection, whether studied using cylinder straw inoculum (Scott, 1971) or spore suspensions (Bateman and Taylor, 1976a,b) as inoculum and subsequent microscopic comparisons of infection. Leaf sheaths, although not possessing cultivar-related differences in resistance, were more resistant than coleoptiles. Hypersensitive reactions on leaf sheaths have been observed but are not related to differences in seedling resistance.

8.2 METHODS

8.2.1 Cultivar growth room evaluations

8.2.1.1 Preliminary evaluations

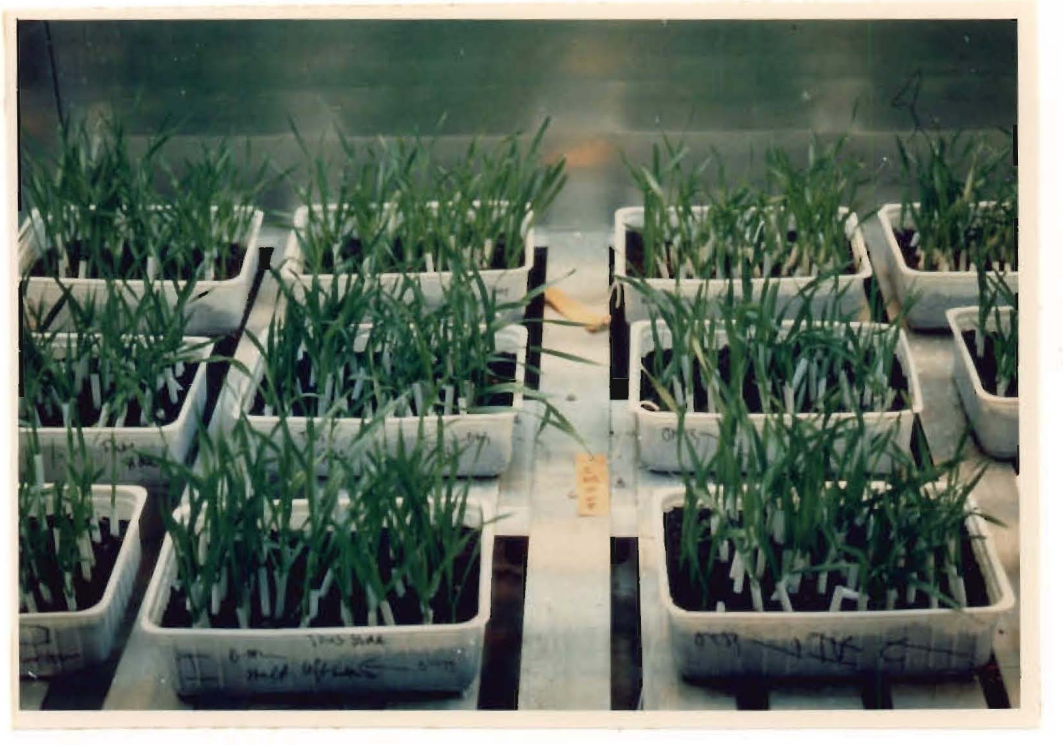
Preliminary trials were undertaken in environmentally controlled growth cabinets on two shelves one above the other (Fig. 8.1). Wheat and triticale cultivars were evaluated in a chamber set at 8.5°C, an average of the 7-10°C optimum described for wheat by Lange-de la Camp (1966). Triticale temperature optima are undescribed. Barley and rye cultivars were evaluated in a separate chamber set at 14°C. This incorporates the barley optimum of 14-15°C and the rye optimum of less than 15°C, but greater than 10°C (Lange-de la Camp, 1966). Relative humidity was kept at 60% and the light regime was maintained at 10h day/14h night as suggested by Brown *et al.*, (1984).

These growth chambers had two major disadvantages:-

1. Plant watering had to be undertaken by hand. There was no allowance within the chamber for automatic trickle irrigation. Although watering was done with care, possibilities of inoculum splash dispersal had to be considered, particularly from top to bottom shelves.

2. As the plant foliage increased, especially that of barley and rye, light intensity on the bottom shelf reduced to half that of the top shelf (measured using a photographic light meter). Disease severity scores of plants from the lower shelf were significantly lower than those from the top.

Fig. 8.1 Preliminary growth room trial, showing straws around seedlings.
The soil level was raised in subsequent trials to just below the tops of the plastic straws.



Subsequent tests were therefore undertaken in an environmentally controlled growth room at DSIR, Lincoln. A computer-controlled trickle irrigation system was installed to ensure splash dispersal of inoculum did not occur. For some small trials, pots were placed in a tray of water. The main disadvantage of the growth room was that all plants had to be at the same temperature. A temperature of 10°C was considered suitable. Relative humidity and the day/night regime was kept as before.

Cultivars evaluated were the same as those in the field trial but without N8020, as no more seed was available. Dominant seed was vernalised for one month at 5°C, before sowing.

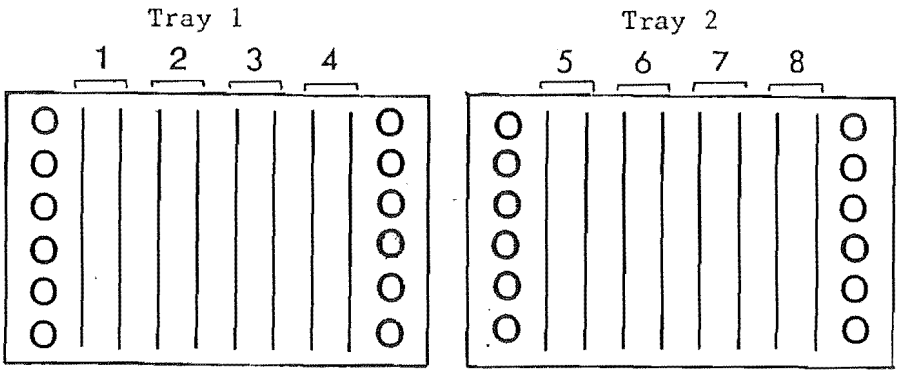
8.2.1.2 Experimental design:

The trial was split into two halves for ease of handling. The wheat and triticale cultivars were evaluated in a 'nursery' together and set up two weeks prior to the barley and rye cultivars. The two nurseries were maintained alongside each other in a controlled growth room. A randomised complete block design was used for each nursery with four replicates, each comprising three blocks. Two plastic trays were used per block and there were two rows of each cultivar with six seeds per row. Four cultivars were sown per tray. In the wheat/triticale trials, the ninth cultivar was split between the two trays (Fig. 8.2). One row of the oat cultivar Omihi was sown along the outer sides of each block as a buffer. Trays were maintained in a glasshouse until germination occurred and seedlings had reached G.S. 12-13. Three treatments were then applied per replicate, these being FE inoculum, SF inoculum and an uninoculated control. Three individual isolates per inoculum type, effectively one isolate per four seedlings, were applied as sub-treatments.

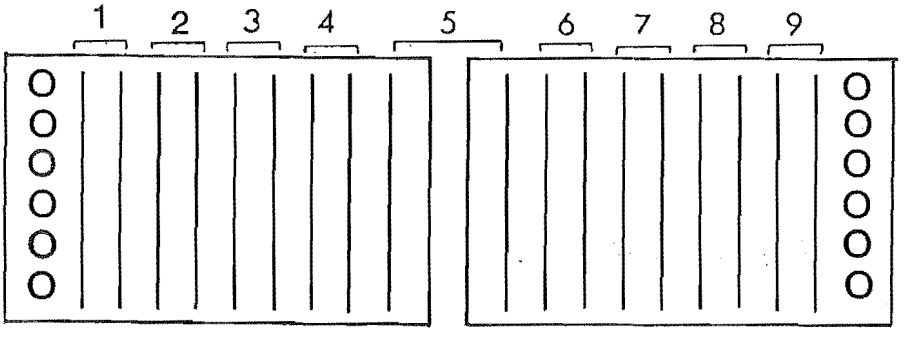
At the time of inoculation, only one SF isolate was available in sufficient quantity for use as inoculum so a repeat of the SF treatments was undertaken when three isolates had been grown up. In this second trial, the cultivars were again split into two nurseries but with only six blocks in each. Four blocks were treated with SF inoculum and the other two were left as uninoculated controls.

Fig. 8.2 Positions of seeds in growth room trays

BARLEY/RYE



WHEAT/TRITICALE



O = Oat seed

Inoculum

Main trial:

FE isolates: K1, K16 4, and K26 1.
SF isolates: 85/2/1

Second trial:

SF isolates: 85/2/4, 86/53/20 and 86/36/2

Dates

Main trial:

Seedlings inoculated:	wheat/triticale	- 1-2/2/86
	barley/rye	- 17-18/2/86
Plants scored:	wheat/triticale	- 21-23/3/86
	barley/rye	- 6-7/4/86

Second trial:

Seedlings inoculated:	wheat/triticale	- 5/4/86
	barley/rye	- 8/7/86
Plants scored:	wheat/triticale	- 20/5/86
	barley/rye	- 6-8/9/86

Randomisations

Wheat/triticale cultivar randomisations across blocks from left to right (see also Fig. 8.3):-

A. 8 9 1 3 4 7 6 2 5
B. 7 8 4 9 5 3 1 6 2
C. 3 1 4 5 8 7 2 9 6

where:-

1 = Bounty
2 = Tiritea

3 = Takahe

4 = Otane

5 = Rongotea

6 = Karere

7 = Aranui N13

8 = Salvo 51B

9 = Lasko

Barley/rye cultivar randomisations across blocks from left to right (see also Fig. 8.3):-

1. 3 2 6 5 7 1 4 5

2. 2 3 6 5 7 4 1 8

3. 7 5 4 1 8 3 2 6

where:-

1 = Gwylan

2 = Fleet

3 = Triumph

4 = Kym

5 = Goldspear

6 = Rapaki

7 = Dominant

8 = Rahu

Isolate randomisations:

Isolates were randomised within each cultivar as three sub-treatments (see also Fig. 8.3).

Isolate randomisation 1.

CULTIVARS	A	B	C	D	E	F	G	H	I	
ISOLATES	3 2 2 2 3	1 3 2 1	2 1 3 3	1 1 1 2 3						
	3 2 1 3 1	1 1 3 1	3 2 1 2	3 2 3 2 3						
	1 2 2 3 1	2 3 3 3	3 1 1 2	1 2 3 1 1						
LEFT	1 3 3 2 2	3 2 2 3	2 2 3 3	2 2 1 2 3	RIGHT					
	2 1 1 3 2	3 1 1 2	1 2 3 2	1 2 3 2 3						
	1 3 1 1 2	3 2 1 2	1 2 3 3	1 1 3 1 1						

Isolate randomisation 2.

CULTIVARS	A	B	C	D	E	F	G	H	I	
ISOLATES	3 2 2 2 1	1 1 2 3	2 3 1 3	3 2 1 1 2						
	1 3 1 1 1	3 1 2 2	3 3 1 2	3 3 2 3 1						
	1 2 2 3 1	2 1 2 3	1 3 2 3	1 1 2 3 1						
LEFT	1 1 3 3 2	3 1 2 1	2 3 1 1	1 3 3 2 3	RIGHT					
	2 3 2 3 2	3 3 3 2	1 1 2 1	2 2 1 1 2						
	2 3 1 1 3	2 3 3 3	1 2 2 2	2 3 1 2 3						

Isolate randomisation 3.

CULTIVARS	A	B	C	D	E	F	G	H	I	
ISOLATES	2 2 3 1 2	3 3 2 3	2 2 3 1	1 2 3 2 1						
	3 1 3 1 3	2 1 2 3	3 1 3 3	3 2 3 2 1						
	3 3 3 2 3	1 1 1 1	2 1 1 1	2 2 3 3 1						
LEFT	1 1 3 1 2	1 3 3 3	1 2 1 2	3 3 1 2 2	RIGHT					
	3 1 2 2 3	1 1 3 2	1 3 2 2	1 2 1 3 3						
	2 2 2 1 2	1 2 2 2	1 3 2 2	3 1 1 1 3						

Isolate randomisation 4.

CULTIVARS	A	B	C	D	E	F	G	H	I	
ISOLATES	2 2 1 2 1	2 2 3 2	3 1 1 2	3 2 1 3 3						
	2 3 2 2 3	3 3 3 3	1 1 2 2	1 1 2 3 2						
	2 3 3 1 3	2 1 3 3	2 1 3 3	3 1 3 1 3						
LEFT	1 3 1 3 1	1 1 2 2	2 3 2 2	1 3 1 2 2	RIGHT					
	1 1 1 3 3	2 1 2 3	1 3 2 2	1 2 3 2 1						
	3 1 2 3 2	1 1 2 1	1 2 3 3	1 2 3 1 1						

Fig. 8.3 Randomisations - cultivar growth room trials

wheat/triticale

FE/B3 3	SF/C2 6	CL/C 9	FE/A1 12
SF/A4 2	CL/A 5	FE/A2 8	SF/B3 11
CL/C 1	FE/B4 4	SF/B1 7	CL/C 10
1	2	3	4

REPLICATE

FE fast-even isolates

SF slow-feathery isolates

CL control

barley/rye

SF/A3 3	FE/A2 6	SF/C1 9	CL/B 12
FE/B4 2	CL/C 5	FE/A3 8	SF/C4 11
CL/C3 1	SF/B2 4	CL/B 7	FE/A1 10

A-C cultivar randomisations

1-4 isolate randomisations

slow-feathery

wheat/triticale

SF/A1 1	SF/B2 2	SF/C3 3
SF/B4 4	CL/A 5	CL/C 6

barley/rye

SF/A1 1	SF/B2 2	SF/C3 3
SF/B4 4	CL/A 5	CL/C 6

1-12 BLOCK SAMPLE NUMBERS

For the barley/rye cultivar blocks with only eight cultivars, the above randomisations were again used but with the randomisation of the fifth cultivar eliminated.

Sampling and scoring

Plants were uprooted, and following removal of plastic drinking straws, were washed in water. Plants were then scored using the 0-5 scale.

8.2.2 Cultivar field evaluations

Cultivars evaluated:-

Wheat:	Bounty, Takahe, Otane, Tiritea, N8020, Rongotea
Barley:	Gwylan, Triumph, Fleet, Kym, Goldspear
Triticale:	Salvo, Lasko, Aranui, Karere
Rye:	Rapaki, Dominant, Rahu

Dominant required vernalisation for one month at 5°C before being spring-sown.

Trial sites

One trial was situated at Lincoln and autumn-sown and a duplicate trial was situated at Gore and spring-sown.

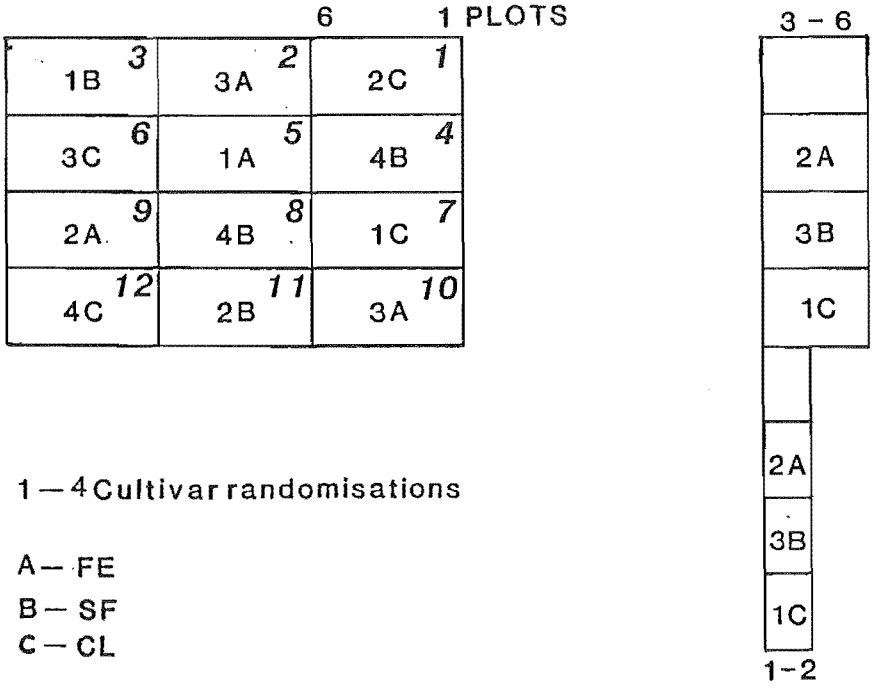
Experimental design

At each site the eighteen cultivars were sown in a randomised complete block design. Three inoculum treatments were applied, these being A; FE isolates, B; SF isolates and C; uninoculated control. In each block, two rows were sown of each cultivar as there were three cultivars per six-row plot. Six plots were required per treatment block of eighteen cultivars.

The main site at Lincoln, measuring 38.4m X 23.4m, allowed four replicates. A fifth replicate was sown at a site removed from the main trial because of land restrictions. This had to be split with one portion containing three blocks of six cultivars and the other containing three blocks of twelve cultivars (Fig. 8.4).

Fig. 8.4 Randomisations - cultivar field trials

Lincoln



Gore

1A 1	3B 2	2C 3	4A 4
3C 5	1A 6	4B 7	2C 8
2B 9	4C 10	1A 11	3B 12
4A 13	2B 14	3C 15	1A 16

1-16 BLOCK SAMPLE NUMBERS

The site at Gore (Fig. 8.5) measured 50.1m X 28.0m, allowing a 4 X 4 array of randomised blocks. A modified latin square design was chosen to accommodate five replications. Blocks were randomly assigned as for a 3 X 3 latin square with a row and column added (Cochran and Cox, 1950). Each treatment was represented in every row and column to eliminate errors among rows and columns.

The trial was sown on a hillside facing the direction of the prevailing southerly wind.

Oats were used as a buffer species, the cultivar Omihi being sown at Lincoln and Ohau at Gore. Buffers were one plot wide around the outside of each trial to prevent edge effects. Between replicates within the trial, buffers were three plots wide as they consisted of two plots of oats mediated by a plot of the wheat cultivar, Rongotea. These wheat plots were sown as control strips to demonstrate any movement of inoculum between replicates.

Cultivar and treatment randomisations

Four cultivar randomisations were computer-generated:-

	PLOT 1	PLOT 2	PLOT 3	PLOT 4	PLOT 5	PLOT 6
1.	3 11 13	5 14 16	1 18 7	10 12 15	2 6 4	8 17 9
2.	8 17 16	18 15 10	3 11 14	6 7 9	1 2 12	13 5 4
3.	16 17 13	10 5 3	11 6 2	12 18 4	14 1 8	7 9 15
4.	8 9 10	13 4 18	3 2 5	7 14 16	12 6 1	11 15 17

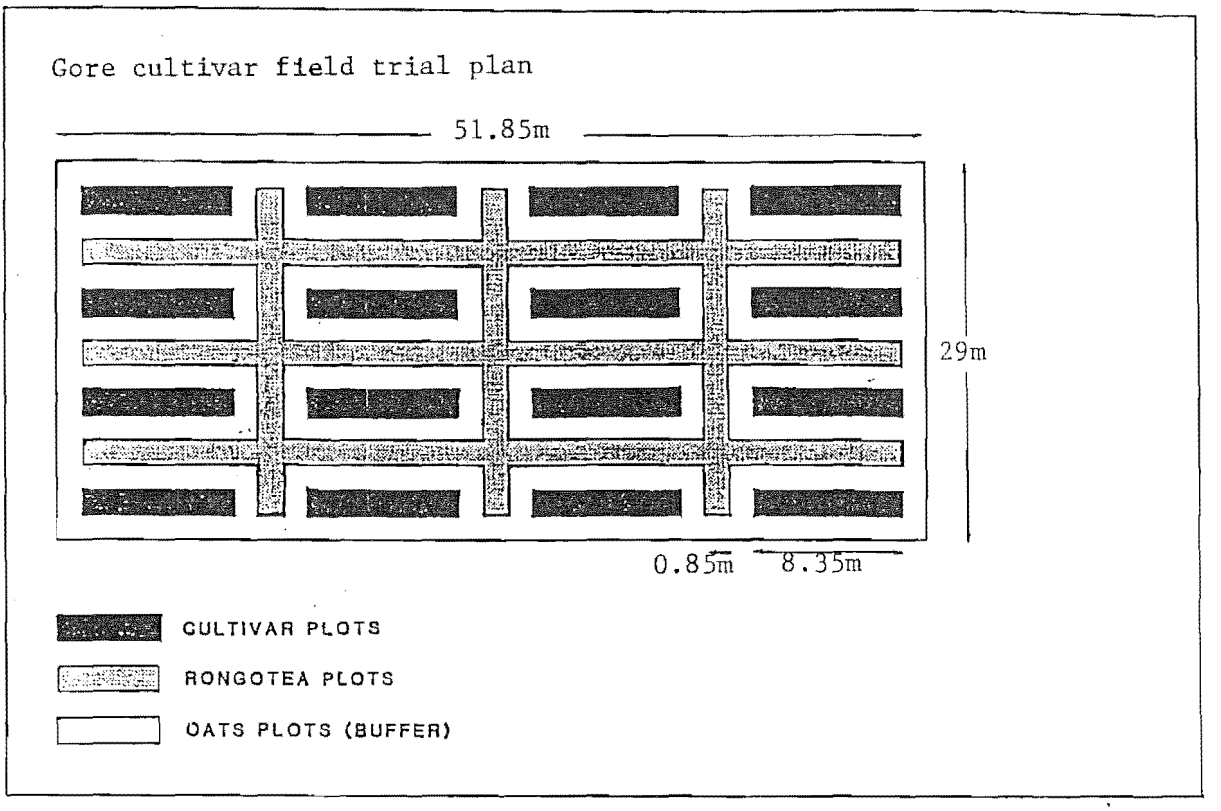
These cultivar blocks were randomised across each trial (Fig. 8.4).

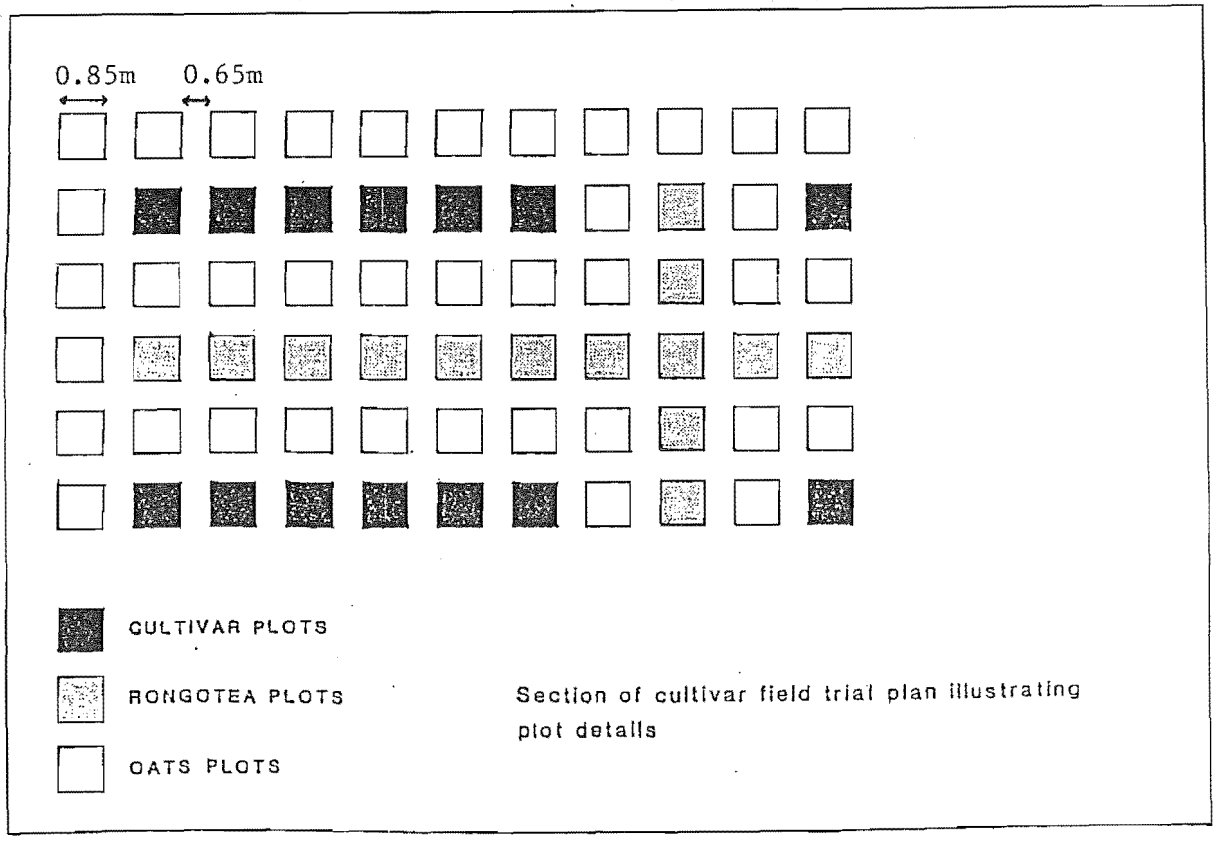
Inoculum

FE isolates: K16 4, K23 1, K1 bulked together.
SF isolates: K16, K13, 85/6/1 bulked together.

Isolates were applied in the form of colonised oatgrains at the rate of 15 g m^{-2}.

Fig. 8.5





Section of cultivar field trial plan illustrating plot details

Sampling and Scoring

Twenty tillers per cultivar were collected randomly from each block at G.S 73-77 and infection scored on the 0-8 scale. Thirty tillers were also collected from each of the Rongotea control plots within the oats buffer and infection was scored as present or absent.
(ref. section 2.2.7.1)

Dates

Drilling:	Lincoln	28/5/85	main plot
		30/5/85	5th replicate
	Gore	25/9/85	
Inoculation:	Lincoln	31/7/85	
	Gore	28/10/85	
Sampling:	Lincoln	10-15/11/85	cultivars
		16/11/85	Rongotea buffer plots
	Gore	6-9/1/86	

Isolations

Isolations were made from Rongotea buffer plots and from rye and triticale cultivars found infected in plots treated with FE isolates in the Lincoln trial.

8.2.3 *Hordeum bulbosum* growth room evaluations

***H. bulbosum* lines:**

51, 2920/4, 2929/1, 2951/4, 2951/5, 2951/6, 2951/7, 2951/8, 2951/9, 2984, 3685, 3811/3, 3811/5, 3811/6, 3811/7, 3816.

Treatments

FE isolate: K16 4

SF isolates: 86/36/2, 86/36/4, 86/36/5, 86/36/6

Experimental design

Bulbs were sown 3-6 per pot and inoculated with treatment isolates using the straw technique. One pot of each line was used per treatment.

Scoring

Plants were scored on a scale of 0-5, using a modification of the leaf infection method. *H. bulbosum* stems are thick and quite succulent. Scores of 1 and 2 were given to plants with infection on the first or second leaves. Scores of 3-5 described disease severity in the central thick stem and was dependent upon the proportion of the stem infected.

Dates

FE treatment

Bulbs inoculated: 27/2/86
Plants scored: 17/4/86

SF treatment

Bulbs inoculated: 27/2/86
Plants scored: 25/4/86

8.2.4 Evaluation of wheat breeding lines

8.2.4.1 Field evaluation

Lines evaluated are listed in Table 8.1.

Table 8.1 Wheat lines from DSIR gene bank

1. 85 SL 6	CIG.2787	*Elymus giganteus* Vahl. X BC3-breadwheat (a standard CIMMYT stock breadwheat)
2. 85 SL 12	CIG.3758	
3. 85 SL 14	CIG 4563	
4. 85 SL 17	CIG 4839	
5. 85 SL 18	CIG 5069	
6. 85 SL 22	CIG 5198	
7. 85 SL 23	CIG 5644	
8. 85 SL 24	CIG 5650	
9. 85 SL 26	CIG 6142	
10. 85 SD 3,	AW84, plot7708, (C1077..X C2429..) X C253	selected breadwheat types of French origin from the DSIR autumn wheat programme
11. 85 SD 4,	AW84, plot7710, (C1077..X C2429..) X C253	
12. 85 SD 5,	AW84, plot7713, (C1077..X C2429..) X C253	
13. 85 SD 6,	AW84, plot7714, C4407.5.5 X(C1.6.5 X C8.2.4)	
14. 85 SD 7,	AW84, plot7715, C4407.5.5 X(C1.6.5 X C8.2.4)	
15. 85 SD 8,	AW84, plot7716, C45.6..[VPM(VOSS X PAP)]R22	
16. 85 SD 9,	AW84, plot7717, C45.6..[VPM(VOSS X PAP)]R22	
17. 85 SD 10,	AW84, plot7725, [(RESCLER X C2419)R8.4]VC70.78	
18. 1-9	*Triticum compactum Host "American Club" cultivated hexaploid*	
19. 1-11	*T. compactum* spring Gembloux	wheats from DSIR
20. 141 (7-101)	*T. compactum* fetisowii	wheat collection
21. 698 (51-9)	*T. dicoccum* Schrank	
23. 704 (51-15)	*T. dicoccum*	
24. 705 (51-17)	*T. dicoccum*	
25. 706 (51-18)	*T. dicoccum*	
26. 719 (57-9)	*T. dicoccum* var. *semicanum*	
27. 12 (1-12)	*T. compactum* (winter)	
28. 29-16	*T. compactum*	
29. 29-17	*T. compactum*	
30. 33-68	*T. compactum*	
31. 33-69	*T. compactum*	
32. 33-70	*T. compactum*	
33. 33-71	*T. compactum*	
34. 35-71	*T. compactum*	

AW = autumn wheat

Trial site

A strip of land was made available at DSIR, Gore and the lines were spring-sown.

Experimental design

Enough seed was available for one plot per line. The site measured 25m X 3m, allowing a width of only two plots. A buffer was required on either side of the experimental plots to prevent edge effects, so the experimental plots were sown down the centre of the strip of land with

a half plot of oats (cultivar Ohau) on either side. The trial is shown in Fig. 8.18.

A statistically randomised design was not constructed for this trial. The size of the plot of land and the limitation in seed quantities made this difficult. It was important that the grain could be later collected for maintaining stock for breeding programmes. As the trial was situated in Southland it was arranged for a breeder to harvest the grain. If the seeds had been randomised within the plots, harvesting would have been made more difficult.

Inoculum

The same composition of inoculum was used as in the fungicide trial, with each respective treatment being a combination of FE and SF isolates.

Date: Inoculated 22/10/86

8.2.4.2 Growth room evaluation

Inoculum

FE: A. 86/36/6 B. 86/36/5 C. 86/78/21
SF: D. GHRS8/D E. 86/32/3 F. 86/2SC/8

Experimental design

Seeds were sown four to a pot and inoculated with treatment isolates using the straw technique. Control pots of uninoculated seedlings were maintained, and clean straws were placed around some of the seedlings.

Scoring

Plants were scored on the 0-5 scale. (ref. section 2.2.7.1)

Dates

Seedlings inoculated : 21/10/86
Plants scored : 29/12/86

8.3 RESULTS

Table 8.2

ANOVA-growth room trial of wheat and triticale cultivars

SF TREATMENT

Analysis of variance of mean scores

Source of variation	DF (MV)	MS	F PR
Rep. stratum	3	0.1282	
Rep. cultivars.stratum			
cultivars	8	0.7167	0.030
residual	24	0.2689	
Total	32	0.3809	
Grand total	35		

Table of means

Grand mean 1.495

Cultivars	Takahe	1.076	
	Salvo	1.309	
	Lasko	1.368	
	Bounty	1.375	
	Karere	1.483	
	Aranui	1.611	
	Tiritea	1.615	
	Rongotea	1.722	
	Otane	1.892	SED = 0.2117

CV = 20%

FE TREATMENT

Analysis of variance of mean scores

Source of variation	DF (MV)	MS	F PR
Rep. stratum	3	0.9532	
Rep. cultivars.stratum			
cultivars	8	14.5116	<0.001
residual	24	0.4046	
Total	32	3.9313	
Rep. cultivars. isolates.stratum			
isolates	2	0.3054	0.424
cultivars.isolates	16	0.2309	0.820
residual	54	0.3506	
Total	72	0.3227	
Grand total	107		

Table of means

Grand mean 2.529

Cultivars	Lasko	0.882
	Salvo	1.368
	Aranui	1.927
	Bounty	1.927
	Takahe	2.347
	Karere	3.003
	Otane	3.646
	Rongotea	3.764
	Tiritea	3.892

SED = 0.2597

CV = 14.5%

Table 8.3 ANOVA - growth room trial of barley and rye cultivars

SF TREATMENT

Analysis of variance of mean scores

Source of variation	DF (MV)	MS	F PR
Rep. stratum	3	4.6215	
Rep. cultivars.stratum			
cultivars	7	2.5971	0.012
residual	15(6)	0.6557	
Total	22	1.2734	
Grand total	25		

Table of means

Grand mean 1.298

Cultivars	Rahu	0.454	
	Rapaki	0.940	
	Dominant	1.059	
	Fleet	1.241	
	Goldspear	1.507	
	Kym	1.635	
	Triumph	1.741	
	Gwylan	1.809	SED = 0.3306

CV = 36%

FE TREATMENT

Analysis of variance of mean scores

Source of variation	DF (MV)	MS	F PR
Rep. stratum	3	0.1290	
Rep. cultivars.stratum			
cultivars	7	3.4845	<0.001
residual	21	0.3702	
Total	28	1.1488	
Rep. cultivars.isolates stratum			
isolates	2	0.8662	0.033
cultivars.isolates	14	0.3333	0.184
residual	45(3)	0.2351	
Total	61	0.2783	
Grand total	92		

Table of means

Grand mean 1.561

Cultivars	Rapaki	0.747
	Dominant	0.972
	Rahu	1.288
	Fleet	1.674
	Gwylan	1.675
	Goldspear	1.757
	Kym	1.958
	Triumph	2.413

SED = 0.2484

CV = 22.5%

Table 8.4 ANOVA - growth room trial - slow-feathery treatment repeat

WHEAT/TRITICALE

Analysis of variance of mean scores

Source of variation	DF (MV)	MS	F PR
Rep. stratum	3	0.3697	
Rep. cultivars.stratum			
cultivars	8	0.8531	0.093
residual	24	0.4305	
Total	32	0.5361	
Rep. cultivars. isolates stratum			
isolates	2	0.5668	0.430
cultivars.isolates	16	0.4138	0.849
residual	54	0.6614	
total	72	0.6038	
Grand total	107		

Table of means

Grand mean 1.892

Cultivars	Lasko	1.598	
	Karere	1.611	
	Aranui	1.653	
	Otane	1.786	
	Takahe	1.848	
	Bounty	1.890	
	Tiritea	2.125	
	Salvo	2.186	
	Rongotea	2.331	SED = 0.2679

CV = 20%

BARLEY/RYE

Analysis of variance of mean scores

Source of variation	DF (MV)	MS	F PR
Rep. stratum	3	2.0115	
Rep. cultivars.stratum			
cultivars	7	0.5016	0.981
residual	21	2.4580	
Total	28	1.9689	
Rep. cultivars. isolates stratum			
isolates	2	0.4283	0.433
cultivars.isolates	14	1.2153	0.012
residual	47(1)	0.5029	
Total	63	0.6588	
Grand total	94		

Table of means

Grand mean 2.217

Cultivars		Isolates	
Rahu	1.976	1	2.215
Dominant	1.993	2	2.103
Goldspear	2.090	3	2.334
Kym	2.108		
Gwylan	2.302	SED = 0.1773	
Rapaki	2.315		
Triumph	2.434		
Fleet	2.521		

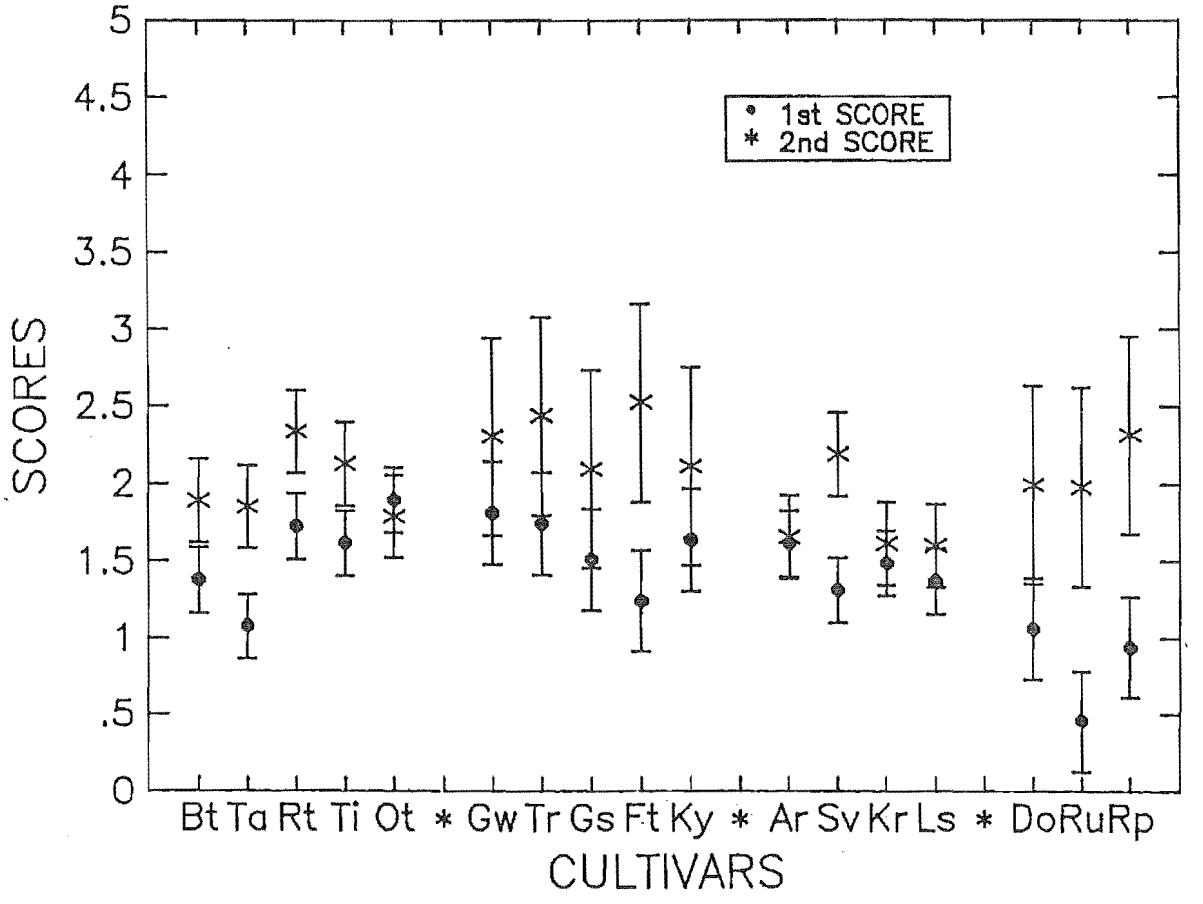
SED = 0.6400

Isolate Cultivars	1	2	3	
Gwylan	2.281	2.250	2.375	
Fleet	2.469	2.313	2.781	
Triumph	2.625	2.438	2.240	
Kym	1.438	2.948	1.938	
Goldspear	2.385	2.031	1.854	
Rapaki	2.719	1.115	3.111	
Dominant	2.083	2.135	1.760	
Rahu	1.719	1.594	2.615	SED = 0.7598

CV = 32%

Fig. 8.6

GROWTH ROOM SCORES OF CULTIVARS INOCULATED WITH SLOW-FEATHERY ISOLATES



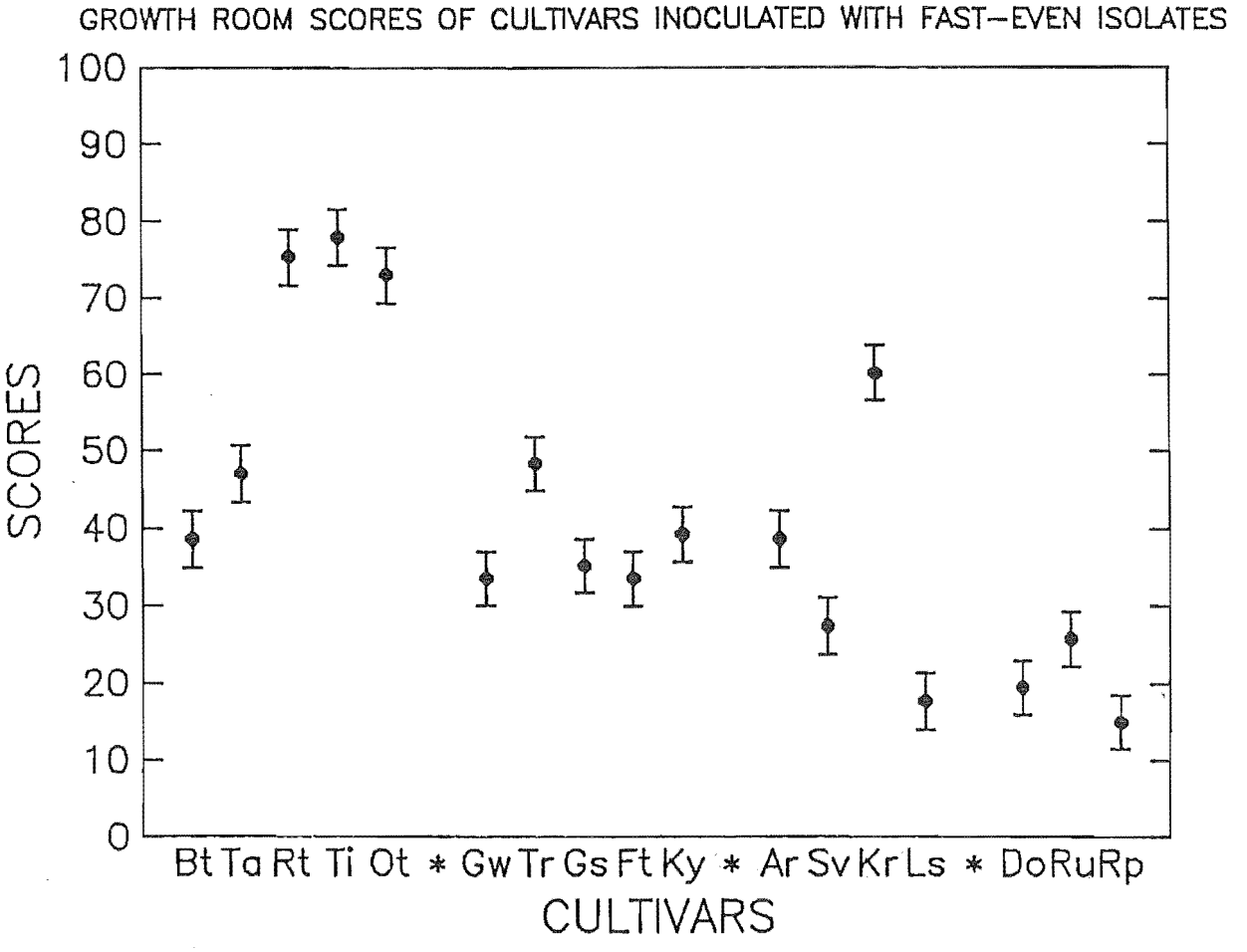
Bt = Bounty
Ta = Takahe
Rt = Rongotea
Ti = Tiritea
Ot = Otane

Gw = Gwylan
Tr = Triumph
Gs = Goldspear
Ft = Fleet
Ky = Kym

Ar = Aranui
Sv = Salvo
Kr = Karere
Ls = Lasko

Do = Dominant
Ru = Rahu
Rp = Rapaki

Fig. 8.7



Bt = Bounty
Ta = Takahe
Rt = Rongotea
Ti = Tiritea
Ot = Otane

Gw = Gwylan
Tr = Triumph
Gs = Goldspear
Ft = Fleet
Ky = Kym

Ar = Aranui
Sv = Salvo
Kr = Karere
Ls = Lasko

Do = Dominant
Ru = Rahu
Rp = Rapaki

Table 8.5 ANOVA - Lincoln cultivar field trial

Analysis of variance of mean scores

Source of variation	DF (MV)	MS	F PR
Rep. stratum	4	573.85	
Rep. fungi. stratum			
fungi	1	25.30	0.660
residual	4	112.44	
Total	5	95.01	
Rep. fungi. cultivar stratum			
cultivars	17	401.49	<0.001
fungi. cultivars	17	107.14	0.037
residual	136	60.32	
Total	170	99.12	
Grand total	179		

Table of means

Grand mean 66.92

Fungi SF 77.05
FE 76.30 SED = 1.581

Cultivars					
	Rahu	62.56	Bounty	76.11	
	Dominant	63.44	Karere	79.13	
	Triumph	71.25	Salvo	74.56	
	Kym	78.47	Tiritea	84.31	
	Gwylan	72.97	Aranui	80.00	
	Rapaki	71.63	Rongotea	81.00	
	Takahe	77.44	Lasko	81.24	
	Fleet	78.38	N8020	82.13	
	Goldspear	80.56	Otane	84.94	SED = 3.473

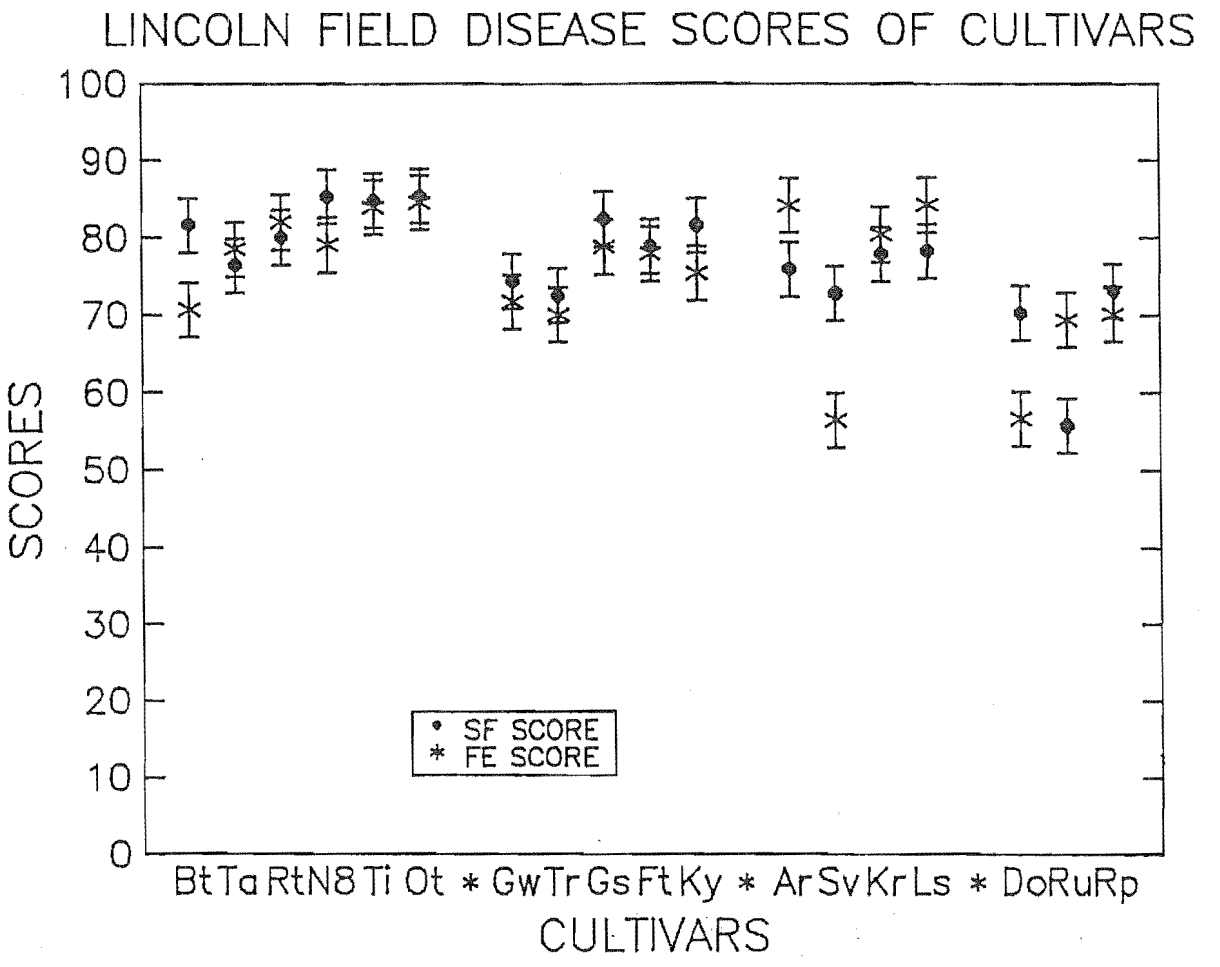
Cultivar Fungi	Salvo	Lasko	Aranui	Karere	Rapaki	Dominant
SF	72.75	78.25	75.88	77.88	73.13	70.25
FE	56.38	84.22	84.13	80.38	70.13	56.63

Cultivar Fungi	Rahu	Bounty	Takahe	Otane	Tiritea	N8020
SF	55.75	81.59	76.38	85.38	84.75	85.25
FE	69.38	70.63	78.50	84.50	83.88	79.00

Cultivar	Rongotea	Gwylan	Triumph	Fleet	Kym	Goldspear	SED = 5.029
Fungi							
SF	80.00	74.31	72.50	78.87	81.57	82.38	
FE	82.00	71.63	70.00	77.88	75.38	78.75	

CV = 10.1%

Fig. 8.8



Bt = Bounty
Ta = Takahe
Rt = Rongotea
N8 = N8020
Ti = Tiritea
Ot = Otane

Gw = Gwylan
Tr = Triumph
Gs = Goldspear
Ft = Fleet
Ky = Kym

Ar = Aranui
Sv = Salvo
Kr = Karere
Ls = Lasko

Do = Dominant
Ru = Rahu
Rp = Rapaki

Table 8.6 ANOVA - Gore cultivar field trial

Analysis of variance of mean scores

Source of variation	DF (MV)	MS	F PR
Rep. stratum	4	1227.1	
Rep. fungi. stratum			
fungi	1	347.6	0.483
residual	4	583.6	
Total	5	536.4	
Rep. fungi. cultivar stratum			
cultivar	17	2426.2	<0.001
fungi. cultivars	17	124.3	0.362
residual	136	113.2	
Total	170	345.6	
Grand total	179		

Table of means

Grand mean 64.08

Fungi SF 65.47
FE 62.69 SED = 3.601

Cultivars	Bounty	30.25	Fleet	70.88	
	Dominant	30.81	Lasko	70.56	
	Aranui	46.94	Goldspear	73.38	
	Rahu	54.75	Triumph	71.97	
	Rapaki	56.25	Rongotea	75.06	
	Salvo	53.63	N8020	73.94	
	Karere	64.00	Kym	79.97	
	Takahe	70.63	Otane	79.44	
	Gwylan	69.44	Tiritea	81.62	SED = 4.758

Cultivar	Salvo	Lasko	Aranui	Karere	Rapaki	Dominant
Fungi						
SF	62.50	72.50	47.25	66.50	66.50	35.50
FE	44.75	68.63	46.63	61.50	61.50	26.13

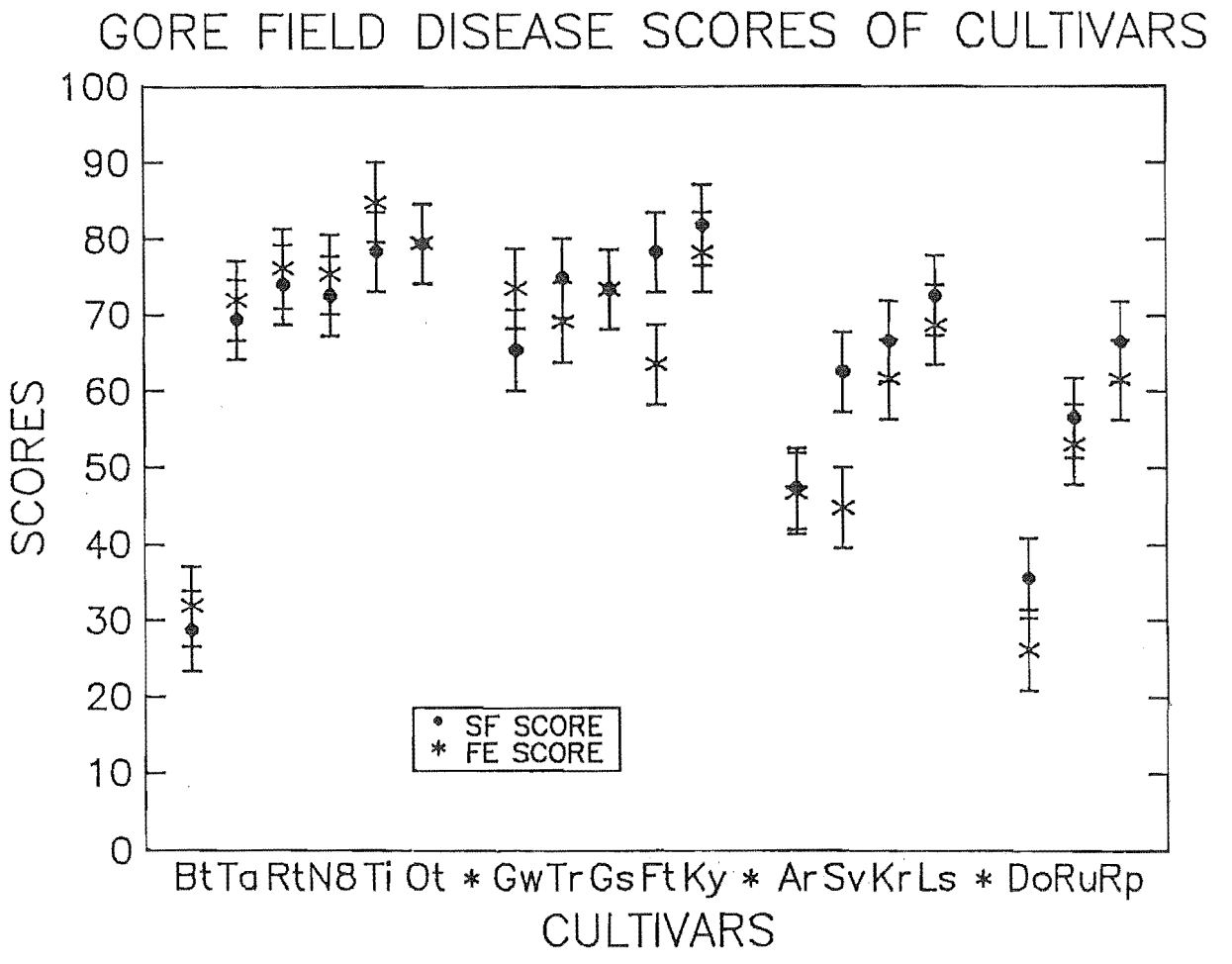
Cultivar	Rahu	Bounty	Takahe	Otane	Tiritea	N8020
Fungi						
SF	56.50	28.63	69.38	79.38	78.38	72.50
FE	53.00	31.88	71.88	79.50	84.86	75.38

Cultivar	Rongotea	Gwylan	Triumph	Fleet	Kym	Goldspear
Fungi						
SF	74.00	65.38	74.88	78.25	81.75	73.38
FE	76.13	73.50	69.06	63.50	78.18	73.38

SED = 7.465

CV = 16.6%

Fig. 8.9



Bt = Bounty
Ta = Takahe
Rt = Rongotea
N8 = N8020
Ti = Tiritea
Ot = Otane

Gw = Gwylan
Tr = Triumph
Gs = Goldspear
Ft = Fleet
Ky = Kym

Ar = Aranui
Sv = Salvo
Kr = Karere
Ls = Lasko

Do = Dominant
Ru = Rahu
Rp = Rapaki

Table 8.7 Morphological types of isolates obtained from treatment plots in Lincoln cultivar trial

Sample	Cultivar	Treatment	Isolate morphology
5/10	Rahu (R)*	FE	SF
5/15	Salvo (T)*	FE	SF
9/9	Rapaki (R)	FE	FE

* R = rye
  T = triticale

Table 8.8 Percentage tiller infection and morphological types of isolates in Rongotea buffer plots of Lincoln cultivar trial

Plot no (Fig. 8.6)	% tiller infection	Isolate types	Plot no	% tiller infection	Isolate types
A1	83.33		G2	86.66	
A2	86.66		G3	90.00	
A3	80.00		G4	66.66	
A4	96.66		G5	76.66	4FE
A5	90.00		G6	73.33	
A6	83.33		H1	90.00	
B1	76.66	1FE	H2	76.66	
B2	96.66	1FE	H3	86.66	1FE,1SF
B3	90.00		H4	70.00	
B4	76.66		H5	56.66	1FE
B5	80.00		H6	60.00	
B6	93.33	1FE	I1	83.33	
C1	70.00		I2	90.00	
C2	73.33		I3	56.66	
C3	60.00	1FE	I4	60.00	
C4	90.00		I5	76.66	
C5	93.33	1FE	I6	46.66	
D1	60.00	1FE	J	76.66	1SF
D2	46.66	1FE	K	90.00	
D3	70.00	2FE	L	80.00	
D4	73.33	2SF	M	53.33	1SF
D5	83.33	1SF	N	63.33	
D6	46.66	1SF,2FE	O	83.33	1SF
E1	93.33		P	43.33	
E2	100.00		Q	50.00	
E3	80.00		W1	23.33	
E4	83.33	1FE	W2	6.66	
E5	83.33		X1	13.33	
E6	90.00	1FE	X2	10.00	
F1	80.00	3FE	Y1	20.00	1FE
F2	76.66		Y2	16.66	1FE
F3	93.33		Y3	13.33	
F4	76.66		Y4	26.66	1FE
F5	73.33		Z1	23.33	1FE
F6	43.33	1SF	Z2	10.00	
G1	86.66	1FE	Z3	23.33	
			Z4	26.66	

Fig. 8.10

Rongotea buffer control plots sampling plan

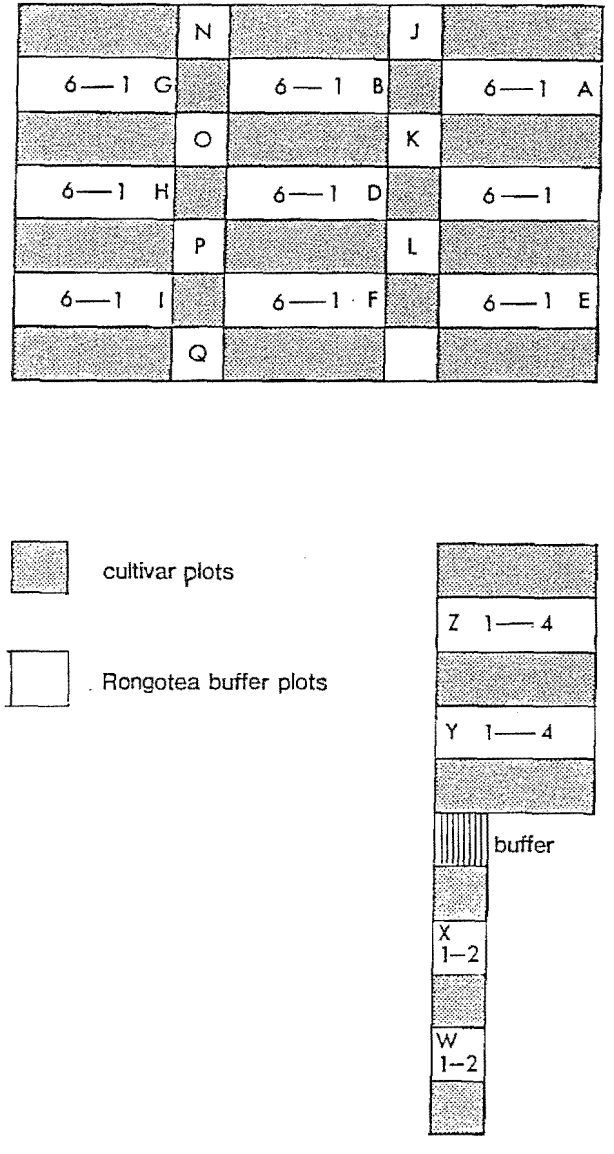


Fig. 8.11

Isolates from Rongotea buffer control plots

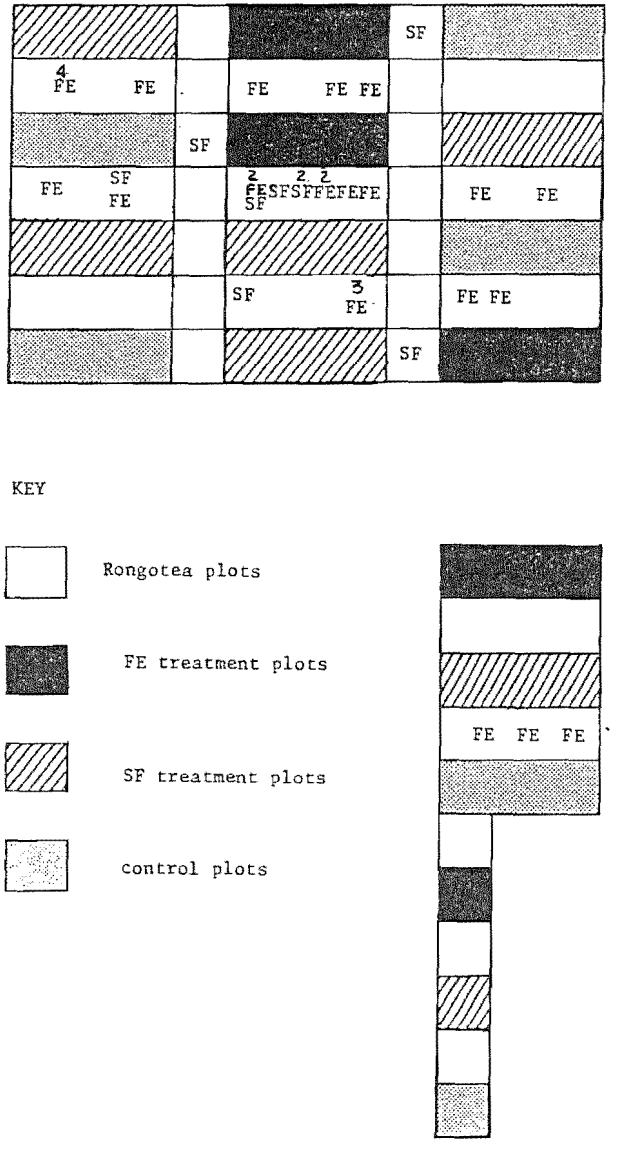


Fig. 8.12

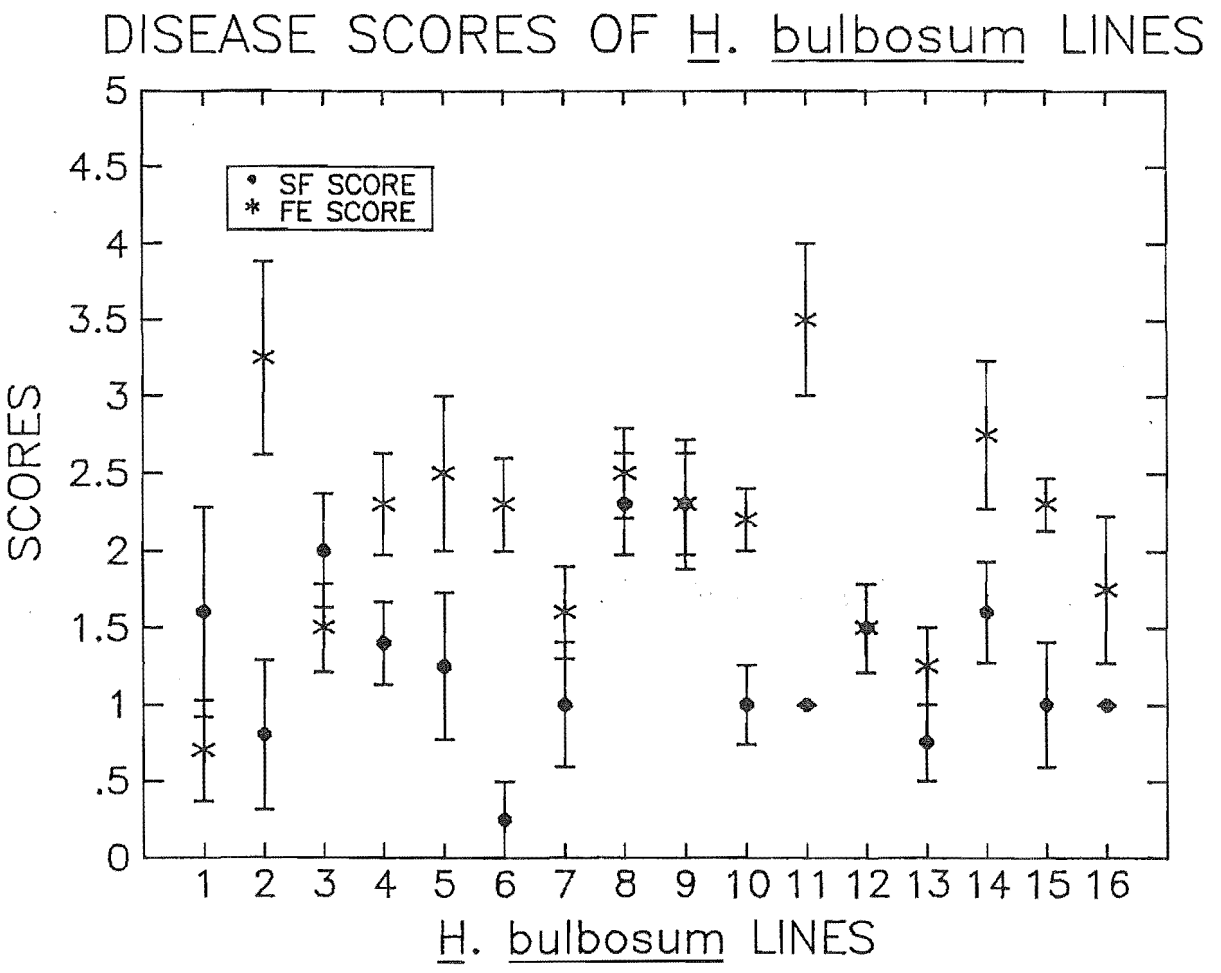

DISEASE SCORES OF H. bulbosum LINES
SF SCORE
FE SCORE
SCORES
5
4.5
4
3.5
3
2.5
2
1.5
1
.5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
H. bulbosum LINES

Table 8.9 Infection scores of *H. bulbosum* lines inoculated with *Pseudocercosporella*

SF inoculum

	H. bulbosum lines	Isolate*	Scores	Mean score	Std Error	Rank
1	S1	4	4, 1, 2, 0, 1	1.60	0.68	7
2	2920/4	2	2, 2, 0, 0, 0	0.80	0.49	3
3	2929/1	2	2, 1, 1, 2, 3, 3	2.00	0.37	15
4	2951/4	1	1, 2, 1, 1, 2	1.40	0.27	11
5	2951/5	1	0, 2, 2, 1	1.25	0.48	10
6	2951/6	1	1, 0, 0, 0	0.25	0.25	1
7	2951/7	3	0, 1, 1, 2	1.00	0.41	7
8	2951/8	1	2, 2, 3	2.30	0.33	14
9	2951/9	1	3, 2, 2	2.30	0.33	16
10	2984	2	1, 1, 2, 1, 1, 0	1.00	0.26	6
11	3685	4	1, 1	1.00	0.00	3
12	3811/3	3	1, 1, 2, 2	1.50	0.29	11
13	3811/5	4	1, 1, 0, 1	0.75	0.25	2
14	3811/6	4	2, 2, 1	1.60	0.33	13
15	3811/7	3	1, 2, 1, 0	1.00	0.41	7
16	3816	3	1, 1, 1	1.00	0.00	3

FE inoculum

	H. bulbosum lines	Scores	Mean Score	Std Error	Rank
1	S1	1, 1, 0	0.70	0.33	1
2	2920/4	2, 3, 3, 5	3.25	0.63	15
3	2929/1	1, 1, 2, 2	1.50	0.29	3
4	2951/4	2, 2, 3	2.30	0.33	9
5	2951/5	2, 2, 2, 4	2.50	0.50	13
6	2951/6	2, 2, 3	2.30	0.33	9
7	2951/7	1, 2, 2	1.60	0.33	5
8	2951/8	2, 2, 3, 3	2.50	0.29	12
9	2951/9	1, 2, 2, 2 ,3, 4	2.30	0.42	11
10	2984	2, 2, 2, 2, 3	2.20	0.20	7
11	3685	2, 4, 4, 4	3.50	0.50	16
12	3811/3	1, 1, 2, 2	1.50	0.29	3
13	3811/5	1, 1, 1, 2	1.25	0.25	2
14	3811/6	2, 2, 3, 4	2.75	0.48	14
15	3811/7	2, 2.5, 2.5	2.30	0.17	8
16	3816	1, 1, 2, 3	1.75	0.48	6

* 1 = 86/36/6
2 = 86/36/4
3 = 86/36/5
4 = 86/36/2

Table 8.10 Field trial of wheat lines

Lines	Scores (frequency)						Sample Number	Disease Severity Index
	0	1	2	3	4	5		
1	0	10	9	3	2	1	25	42.40
2	1	6	5	4	1	3	20	47.00
3	1	8	2	7	6	1	25	49.60
4	1	8	5	1	3	2	20	43.00
5	1	8	6	3	3	3	24	46.66
6	0	7	3	2	4	4	20	55.00
7	1	4	7	6	3	0	21	45.71
8	2	11	6	2	0	0	21	27.62
9	2	11	8	3	7	2	33	44.85
10	3	7	3	12	1	0	26	40.77
11	5	0	2	1	10	1	19	54.74
12	0	0	2	5	13	1	21	72.38
13	1	9	2	2	2	0	26	20.77
14	2	4	4	0	3	0	23	20.87
15	0	3	3	3	11	2	22	65.46
16	0	2	1	7	3	0	23	32.17
17	0	6	5	7	7	0	25	52.00
18	1	17	2	4	1	0	25	29.60
19	3	4	10	9	4	0	30	43.23
20	2	8	5	5	2	1	23	40.00

Wheat lines - field results

The wheat lines are grouped according to mean disease severity and ordered within groups according to increasing variance.

Group 1 - lowest disease severity

A 8. 85 SL 24 CIG 5650
18. 1-9 *Triticum compactum* 'American Club'

B 13. 85 SD 6, AW84, plot7714, C4407.5.5 X(C1.6.5 X C8.2.4)
14. 85 SD 7, AW84, plot7715, C4407.5.5 X(C1.6.5 X C8.2.4)

C 16. 85 SD 9, AW84, plot7717, C45.6..[VPM(VOSS X PAP)]R22

Group 2

A 10. 85 SD 3, AW84, plot7708, (C1077..X C2429..) X C253
20. 141 (7-101) *T. compactum* fetisowii
1. 85 SL 6 CIG.2787
4. 85 SL 17 CIG 4839
19. 1-11 *T. compactum* spring Gembloux
9. 85 SL 26 CIG 6142
7. 85 SL 23 CIG 5644
5. 85 SL 18 CIG 5069
2. 85 SL 12 CIG 3758
3. 85 SL 14 CIG 4563

Group 3

A 17. 85 SD 10, AW84, plot7725, [(RESCLER X C2419)R8.4]VC70.78

B 6. 85 SL 22 CIG 5198

C 11. 85 SD 4, AW84, plot7710, (C1077..X C2429..) X C253

Group 4 - highest disease severity

A 12. 85 SD 5, AW84, plot7713, (C1077..X C2429..) X C253

B 15. 85 SD 8, AW84, plot7716, C45.6..[VPM(VOSS X PAP)]R22

Table 8.11 Overall results for wheat lines

	Field	Growth Room	
		SF treatment	FE treatment
1	42.40		
2	47.00		
3	49.60		
4	43.00		
5	46.66		
6	55.00		
7	45.71		
8	27.62		
9	44.85		
10	40.77		
11	54.74		
12	72.38		
13	20.77		
14	20.87		
15	65.46		
16	32.17		
17	52.00		
18	29.60	23.20	35.00
19	43.23	16.60	62.60
20	40.00	30.00	100.00
21		25.00	40.00
22		32.50	39.20
23		46.40	83.20
24		27.20	34.60
25		23.00	44.20
26		68.60	100.00
27		25.40	32.60
28		20.00	20.00
29		40.00	20.00
30		30.00	40.00
31		20.00	-
32		38.00	41.40
33		26.60	53.20
34		33.20	93.20

Fig. 8.13

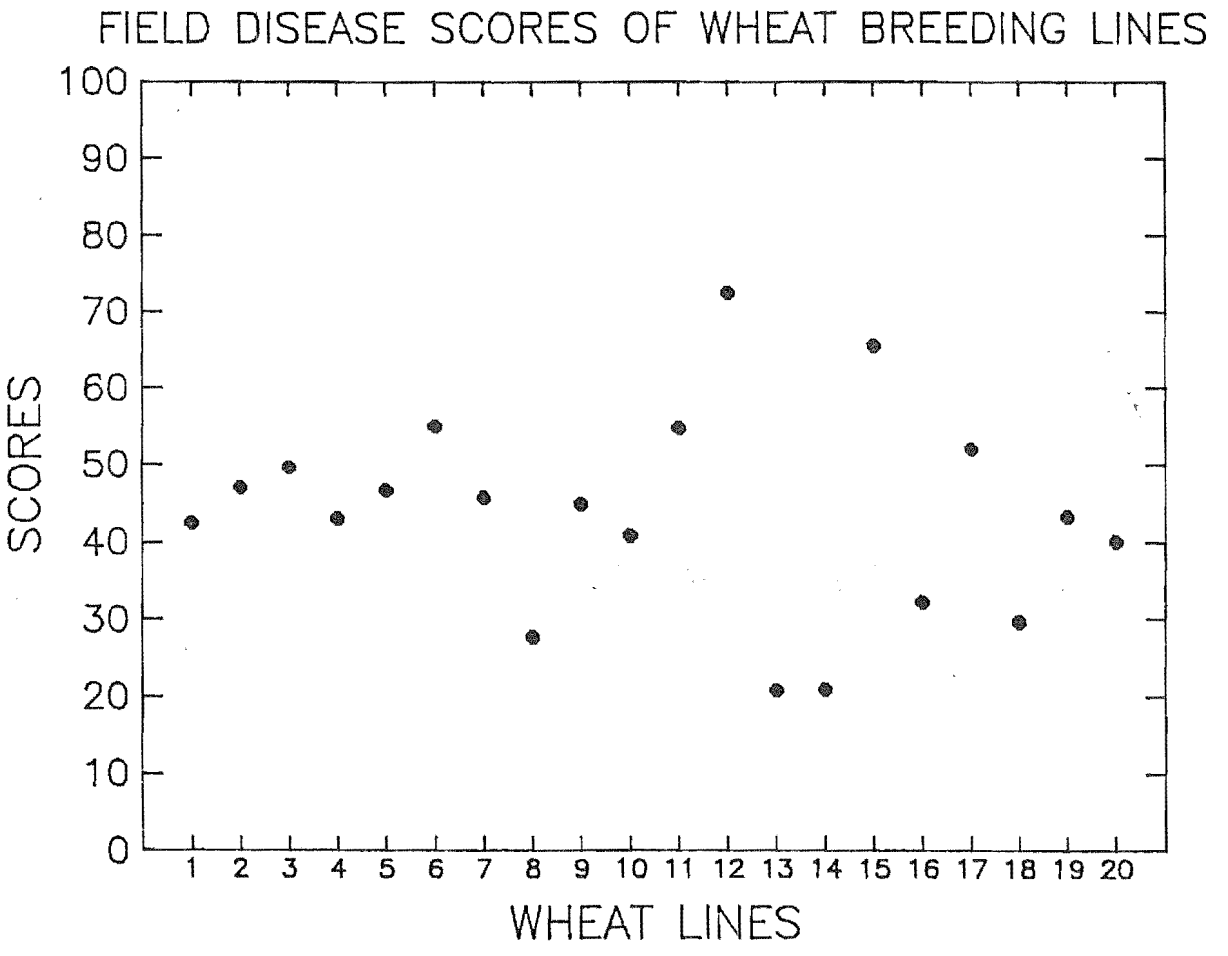
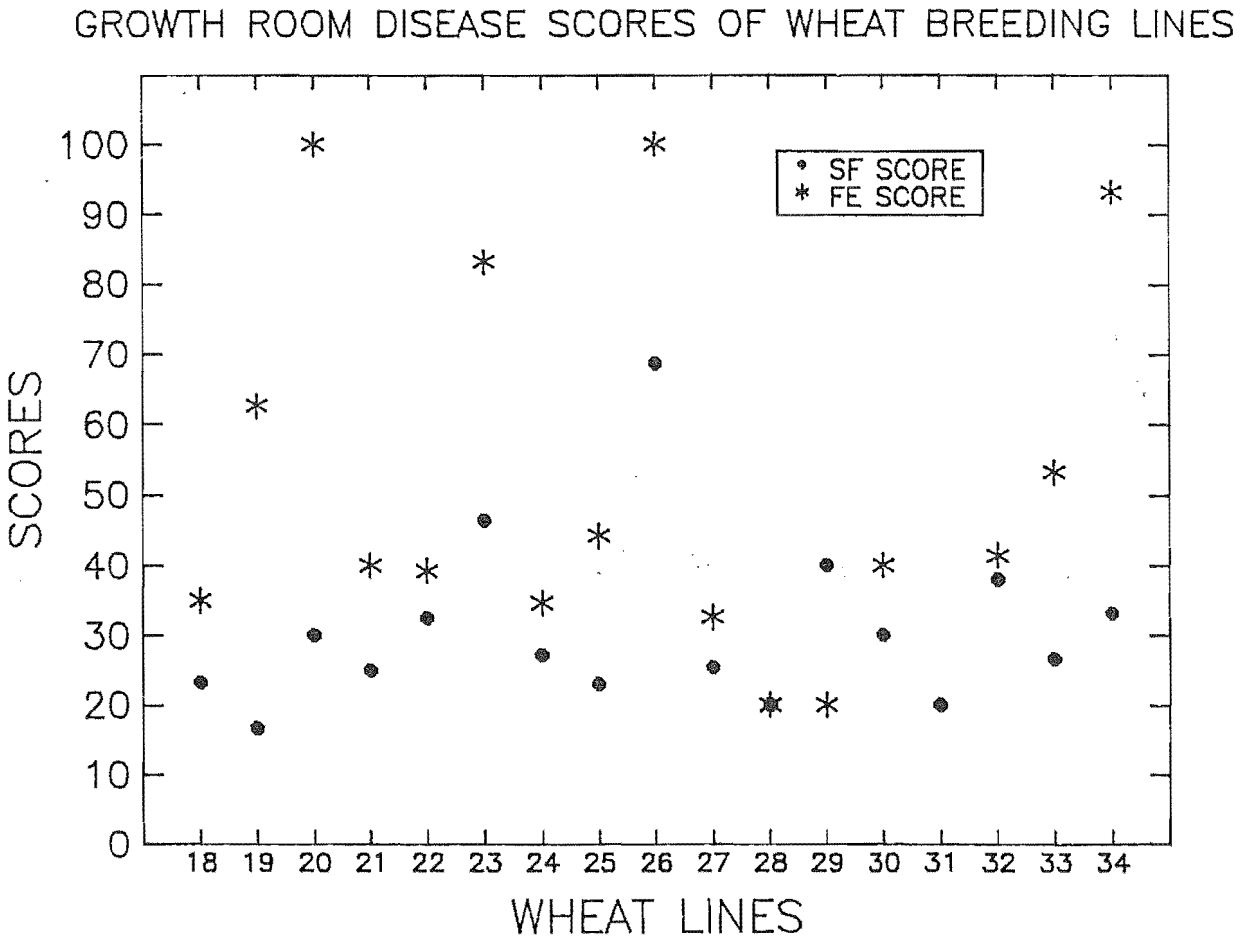

FIELD DISEASE SCORES OF WHEAT BREEDING LINES
SCORES
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
WHEAT LINES

Fig. 8.14


GROWTH ROOM DISEASE SCORES OF WHEAT BREEDING LINES
SF SCORE
FE SCORE
SCORES
0
10
20
30
40
50
60
70
80
90
100
18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34
WHEAT LINES

8.3.1 Cultivar growth room evaluations

No control plants were infected in any trials, so controls were omitted from the statistical analyses. Treatment scores for each trial were analysed separately. As there were numerous missing values, analyses of variance were determined for the means of the infection scores of the four plants comprising a cultivar/isolate subtreatment. Tables of analyses of variance (ANOVAs) and means are presented in Tables 8.2 - 8.6 and graphs of the results are in Figs 8.6 - 8.9.

Wheat/triticale evaluations

Following treatment with FE inoculum, the wheat cultivars Rongotea, Tiritea and Otane, and the triticale cultivar, Karere, had the highest disease scores. All other wheat and triticale cultivars scored much lower with Lasko scoring the lowest. The differences between cultivar scores were highly significant ($P<0.001$), however differences between the three FE isolates ($P=0.424$) and responses of the different cultivars to the different isolates ($P=0.820$) were not. The overall mean disease score was 2.529 with a standard error of 0.26.

There was much less variation between cultivars following inoculation with SF isolates. The first trial, which employed only one isolate, produced significantly different scores ($P=0.03$). The second trial, employing three different isolates, had a higher overall disease severity (1.892) than the first trial (1.495), but produced no significant differences between cultivar disease scores. The relative ranking of cultivars in each of the two trials were very similar. Bounty and Takahe scored lowest overall in the SF trial, which was consistent with their responses to FE inoculum as compared with other wheat cultivars. There were no significant differences ($P=0.430$) between the three isolates in the second trial, nor between the interactions of the isolates with the different cultivars ($P=0.849$).

Barley/rye evaluations

In the barley and rye evaluations, highly significant differences ($P<0.001$) were obtained between scores of cultivars treated with FE inoculum. In contrast to their behaviour on wheat and triticale cultivars, the isolates were significantly different in their pathogenicity ($P=0.033$) although cultivar X isolate interactions were not significant ($P=0.184$). The mean disease score for the rye cultivars was 1.002 and the mean disease score for the barley cultivars was 1.895. Rahu had the highest disease severity (1.288) of all rye cultivars treated with FE inoculum, however this score was still lower than the lowest barley scores (1.674).

In the first trial evaluating effects of SF inoculum, wherein only one isolate was used, significant differences ($P=0.012$) were obtained between cultivars, with a mean disease score for rye cultivars of 0.8177. In the second trial in which three isolates were evaluated, the overall mean disease score for the rye cultivars was 2.095 and that for the barley cultivars was 2.291. Disease severity was much higher in the second trial (2.217) than the first (1.298). There were no significant differences between either cultivars ($P=0.981$) or isolates ($P=0.433$), however there was a significant interaction ($P=0.012$) between the two. In the second SF trial, isolate two produced a higher disease score on Kym than did isolate three, whereas on the rye cultivars Rapaki and Rahu, isolate two produced exceptionally low mean scores. On Rapaki, the difference between scores produced by isolates two and three was nearly three times greater than the standard error.

FE inoculum

Overall, in response to inoculation with FE isolates, the wheat cultivar scores fell into two distinct groups. Rongotea, Tiritea and Otane had severest disease scores, which were also similar to the scores obtained for the triticale cultivar, Karere. Bounty and Takahe had less disease, of a level similar to the barley cultivars and also the triticale, Aranui. The three rye cultivars and the triticales, Salvo and Lasko, were in a group described by generally minor infection.

SF inoculum

In the first SF trial, the wheat, barley and triticale cultivars scored similarly overall, except for the wheat cultivars, Bounty and Takahe, which scored much lower and at a level similar to the rye cultivars. In the second SF trial, the range of disease scores was smaller with Bounty, Takahe and the rye cultivars scoring more similarly to the other cultivars.

8.3.2 Cultivar field evaluations

The main site of the Lincoln trial is shown in Fig. 8.15 and the fifth replicate in Fig. 8.16. Two statistical analyses were made of scores obtained in the Lincoln field trial, the block of four replicates being analysed first and then all five replicates were analysed together. The two analyses were almost identical so the ANOVA of all five replicates was used (Table 8.5).

Fig. 8.15 The main site of the Lincoln cultivar field trial with a block of Rongotea wheat to the left and a treatment block to the right

Fig. 8.16 The fifth replicate of the Lincoln cultivar field trial, with the Poplar shelter belt to the right

As the Gore field trial design was a variation of a latin square, it was not possible to analyse every block as is the case with randomised block designs. There were 16 treatment blocks, with 5 blocks for each of SF inoculum and an uninoculated control, but 6 treatment blocks for FE inoculum. Possible variation across the trial was analysed by first designating rows as replicates and analysing row X column interactions and then by designating columns as replicates and analysing column X row interactions. In the former analysis, blocks constituting the fourth column were therefore omitted, and in the second analysis, blocks constituting the fourth row were omitted. There were no differences between the outcome of these analyses, so a third analysis was performed on 15 of the 16 blocks, arbitrarily designating these as five replicates of three blocks. A randomly assigned FE corner block was omitted from this final analysis (Table 8.6).

The grand mean of disease index scores from the Lincoln trial was 76.67, and that for the Gore trial was 64.08.

In both trials, highly significant differences ($P<0.001$) were obtained between cultivar disease index scores. Interactions between treatments and cultivars were significant for the Lincoln trial ($P=0.037$) but not the Gore trial ($P=0.362$).

In the Lincoln trial, following FE treatment, the lowest scoring of the barley cultivars were Triumph (70) and Gwylan (72) and the highest scoring was Goldspear (79). Of the wheat cultivars, Bounty had the lowest score, which was 71, with the other cultivars scoring between 78 and 85, with Otane scoring the highest. The triticales scored between 80 and 85 except for Salvo which was the most resistant with a score of only 56. The rye cultivar, Dominant, scored 57 which was considerably lower than scores for the other ryes which were 69 and 70.

Following SF treatment, of the wheat cultivars, Takahe had the lowest score, which was 76, whereas the other wheat cultivars scored from 80 to 86, with Otane scoring the highest. The barley cultivars scored slightly higher than they did following FE treatment, except for Kym which scored 82 following SF and only 75 following FE treatment. The

triticales scored from 73 - 78 and the ryes, Rapaki and Dominant scored 73 and 70, respectively. The rye cultivar, Rahu, had the lowest score (55.75) over the whole trial following SF treatment.

In the Lincoln trial, differences between FE treatment and SF treatment scores were greatest for Bounty, Aranui, Dominant and Rahu.

In the Gore trial, following FE treatment, scores for the wheat cultivars ranged from 72 - 75, except for Bounty which was the most resistant, with a score of 32. The barley cultivars scored from 65 to 81 with Fleet being the most resistant and Kym the most susceptible. The cultivars Aranui and Salvo, scoring 47 and 45, were the most resistant of the triticale cultivars, as Karere and Lasko scored 62 and 69, respectively. Of the rye cultivars, Dominant scored only 26, and was therefore the lowest scoring of all cultivars, whereas Rahu and Rapaki scored 53 and 62.

Following SF treatment, the wheat cultivars scored just slightly lower than they did following FE treatment. The barley cultivars varied in their scores, with Gwylan scoring lowest with 65 and Kym scoring highest with 82. The triticale cultivars scored just slightly higher than they did following FE treatment, except for Salvo, which scored 63, as compared with 45 following FE treatment. The rye cultivars scored from 36 - 37 which was higher than the range following FE treatment.

The greatest differences between scores for each treatment were obtained for Fleet, Salvo, Rapaki and Dominant.

The three rye cultivars had scores amongst the lowest in both the Lincoln and Gore trials, following either inoculum treatment. Triticales comprised the next group of low scoring cultivars for SF treatment. For the FE treatment scores, however, the triticales had low scores in the Gore trial, similar to those obtained in the SF treatment, but had amongst the highest scores in the Lincoln trial. Barley scores in both the trials were lower in species rank for SF than FE treatments. The wheats comprised the top-scoring cultivars in the Lincoln trial, alone in SF treatments, and together with the

triticales in the FE treatments. In the Gore trial, the wheats varied widely across the range of scores in the SF treatments, with Bounty scoring the lowest of all cultivars. In the FE treatments, Bounty again scored very low, but the other wheat cultivars scored higher than the triticale and rye cultivars, and slightly higher than the barley cultivars.

Isolations were made from some samples of rye and triticale found infected in FE inoculated plots in the Lincoln cultivar trial and two of the three isolates obtained were SF (Table 8.7).

Scores and isolations from Rongotea plants in buffer control plots were only made from the Lincoln site. Tiller infection percentages (Table 8.8) within the main trial site were high, ranging from 44-100% but within the fifth replicate were lower and ranged from 7-27%. Of 36 isolates obtained from these plots, 27 were of FE colony morphology and 9 were of SF colony morphology.

8.3.3 *H. bulbosum* growth room evaluations

The *H. bulbosum* lines (Fig. 8.17) generally scored higher following inoculation with FE than with SF isolates and therefore behaved like triticales (Table 8.9, Fig. 8.12). Lines 51 and 3811/5 had the lowest infection following inoculation with isolates of either pathotype, hence were the most promising as a source of eyespot resistance. Lines 2951/8 and 2951/9 had the highest average infection for both types of isolates. All other lines had reasonable levels of infection although were more susceptible to FE isolates. The lowest score was produced by line 2951/6 following infection with SF isolates, however, the score following the FE treatment was much higher. Lines 2920/4 and 3811/3 scored the highest and this was following FE treatment. Line 2929/1 was the only line (other than 51) to score higher for SF than FE treatment.

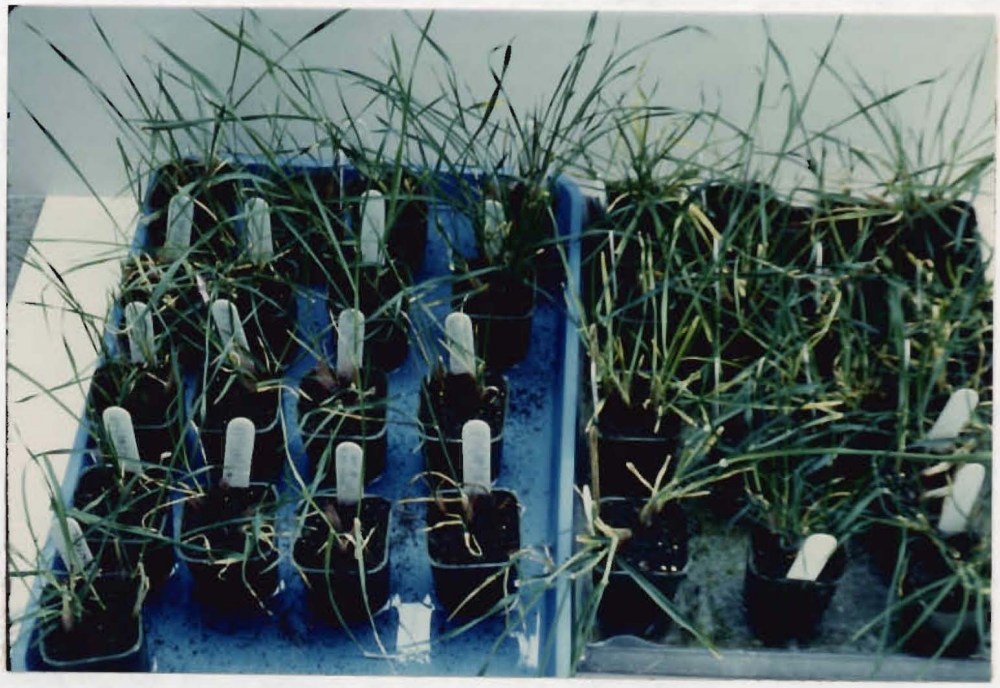


Fig. 8.17 *H. bulbosum* plants in the growth room trial.

Fig. 8.18 The wheat breeding lines sown in Southland, surrounded by half plots of oats.

8.3.5 Evaluation of wheat breeding lines

The wheat breeding lines (Fig. 8.18) varied in their susceptibilities to infection by *Pseudocercosporella*. The 20 lines assessed in field trials could be described in three broad classes (Table 8.10). Lines 8, 13, 14, 16 and 18 had the lowest scores, however the standard error within each group of lines increased respectively. Lines 13 and 14 had low scores of 1.039 and 1.044, with standard errors of 0.2448 and 0.2085 respectively. Lines 12 and 15 had the highest scores (3.62 and 3.27 respectively), however, lines 6, 11 and 17 also scored moderately highly, (2.6 - 2.8), with lines 6 and 11 having large standard errors, 0.3618 and 0.4177 respectively. The other lines scored midway between these groups and had similar standard errors except for lines 4 and 5 which had reasonably high standard errors (0.3346 and 0.3050). Lines 18, 19 and 20, which were also assessed in a growth room trial for responses to the different *Pseudocercosporella* pathotypes, in each case scored lower in response to the SF infection in the growth room (Fig. 8.14) than to the mixed inoculum in the field trials (Fig. 8.13, Table 8.11). In contrast to this they each scored higher for the FE treatment than in the field trials. Lines 21-34, which were only evaluated in growth room trials, were all more susceptible to FE isolates than SF, except for line 29, the scores of which were ranked in reverse. Line 26 was the most susceptible line to both pathotypes. No score was obtained for the responses of lines 28 and 31, to FE infection. Lines other than 23, 26, 28, 31, 32, 33 and 34, showed some level of resistance to both pathotypes with scores ranging from 1 to 2.

8.4 DISCUSSION

8.4.1 Inoculation

Artificial inoculum in field trials must be uniform and produce a uniform response in the absence of other treatments. Infection in the field is presumed to be by conidia, vegetative hyphae and possibly also ascospores. If it is assumed that infection by a vegetative hypha or a germinating spore produces the same host response, the type of inoculum chosen for an experiment should not matter. Also, disease

pressure has been demonstrated to be more dependent on weather conditions than on the amount of available inoculum (Cox and Cock, 1962; Hollins and Scott, 1980). Disadvantages in using conidial suspensions include the difficulty in producing conidia in large quantities and the dependence on suitable weather conditions following inoculation. Isolates exhibiting reduced sporulation are more difficult or impossible to test for pathogenicity in this way, yet the isolates may still be efficient in mycelial infection. In growth room trials, it is more difficult to maintain conidia than mycelium on seedling stems, and they are likely to be splashed between stems during application. Bateman and Taylor (1976a,b) placed plastic tubes over seedlings to enclose the site of inoculation primarily to maintain humidity, but these would also serve a role in preventing cross-contamination. Such an enclosed environment could interfere with the gas composition and alter responses to infection. Conidial suspensions may allow multiple infection points which complicates scoring. In his colonised wheat straw technique, Macer (1966) suggested that additional soil around straws would ensure no exposure to light, preventing sporulation, hence maintaining a constant source of inoculum. A disadvantage with colonised wheat straws is that they prevent the fungus from initially competing with other soil microbes, thus providing a much higher inoculum potential than would perhaps be the case in the field. This problem is retained by using plastic straws.

As the quantity of PDA applied to seedlings is minimal, it would provide little additional substrate for inoculum growth compared with wheat straws and would also be more uniform. The hyphal plug technique also overcomes the problem of numerous simultaneous infections which occur with colonised wheat straws. The hyphal plugs are maintained in a localised position, however this could increase the infection potential. It was hoped that the direct application of hyphae combined with additional soil around the straws would allow for infection before sporulation.

8.4.2 Infection assessment

The methods employed for scoring infected plants proved successful as significant differences between infection means were obtained. Analyses were made of unadjusted scores and also of the squares of the scores. When the range of scores on the 0-3 scale is used, scores may be squared to accentuate differences. The scales of 0-5 and 0-9 are more specific in what they describe and squaring of the scores increased the within-cultivar variation, rather than that between, and hence obscured differences.

8.4.3 Growth room trials

Growth room trials showed FE isolates to be more pathogenic on wheat cultivars than rye. This is in accordance with the expected association of FE morphology with W-type pathogenicity. The first SF trial using one isolate produced low disease scores, with rye plants being infected slightly less than wheat, however in the second trial, employing three isolates, high disease severities were obtained and there were no significant differences between the cultivars. These scores are also in accordance with the expected association of SF morphology with R-type pathogenicity. The possibility that R-type isolates have been selected in the U.K. by an increase in the amount of barley sown, is supported by the present scores following treatment with SF isolates but not by the high scores which were also obtained after FE treatment. These barley cultivars are very susceptible to both morphological types of *Pseudocercosporella*.

The presence of cultivar X isolate interactions is not unexpected as both different isolates and cultivars are likely to possess a range of different virulence and resistance genes. Interactions could also be explained by the variation in seed germination that was obtained. As all seedlings were inoculated at the same time there was some variation in the development of the leafsheaths around the coleoptiles. Host X isolate interactions have been reported by other workers. Scott *et al.*, (1976) obtained interactions in each of three experiments. Bateman and Taylor (1976b) suggested that variation obtained in infection within seedlings of the same cultivar may result

from positioning inoculum on the coleoptiles of some seedlings and the leaf sheaths of others. The significance of the coleoptile in the establishment of infection has been shown (Bateman and Taylor, 1976a,b). Plants with coleoptiles were significantly more infected than those without and infection can occur directly from appressoria produced by surface hyphae. Subsequent infection of the first leaf sheath occurs primarily from stromata developed between the coleoptile and leaf sheath. It was suggested that the combination of increased inoculum potential provided by stromata and early senescence of the coleoptile probably assists fungal penetration of the leaf sheath. The temperature optima described by Scott (1971) are not strict but likely to vary between isolates. Variation in optimal temperatures would be obscured in the field where a range of temperatures are experienced.

8.4.4 Field trials

In the field, cultivar responses were variable. The Gore trial produced a greater range of disease scores in plots of either treatment than the Lincoln trial, however the maximum scores obtained were similar at both sites. Considerably lower scores were obtained in Gore for some cultivars, including the wheat cultivar Bounty, which has Capelle-Desprez resistance, and the rye cultivars. The major difference between the Gore and Lincoln site is the sowing time. Southland crops, when spring-sown, have a much shorter growing season than those autumn-sown in Canterbury. Reduced eyespot incidence which commonly results from late sowing of winter wheat is probably a consequence of delayed crop development (Hollins and Scott, 1980). In Canterbury, the longer growing season prolongs the early growth stages of the plants and in this, perhaps also maintains the more susceptible coleoptile stage for a time long enough to allow deep-seated infection to occur. Overall disease severity was higher at Lincoln than Gore. This is in contrast to the expected, as eyespot is frequently a major problem in Southland crops and of minor severity or absent in Canterbury. Hollins and Scott (1980) also suggested that delayed exposure to inoculum would have an effect in reducing the development of eyespot. Inoculum must not have been applied to the field trials at Lincoln at an appropriate time to mimic natural inoculum. It is

possible that the natural infection period at Lincoln is much later, perhaps mid-late spring, than the late winter - early spring application of trial inoculum and for this reason, cultivars exhibiting some level of resistance in the Gore trial could also be expected to exhibit it in the naturally-infected crops at Lincoln. Differences between cultivars are established during the early stages of infection and if assessment is left late, differences between the cultivars may become reduced as the mean number of uninfected leaf sheaths decreases (Scott, 1971).

The infection time is dependent upon factors which differ between the Canterbury and Southland environments. Southland has a wet, cold climate in contrast to Canterbury. During the time of the trials, Southland experienced nearly double the number of wet days than Lincoln (Table A6.1) and as the growing season was shorter, effectively had the same quantity of rain as Lincoln. Hollins and Scott (1980) found infection to be more closely correlated to the number of wet days than total rainfall. Temperature was not markedly different between the two sites (Table A6.2) and is therefore not considered to have played a role in producing the different disease pressures and in any case, Scott (1971) determined that temperature is not a critical factor when comparing cultivars.

8.4.5 *H. bulbosum* and wheat breeding line evaluations

A line requires a level of resistance to both pathotypes of *Pseudocercosporella* before being considered for inclusion in a breeding programme. The *H. bulbosum* lines differed from the cultivated *Hordeum vulgare* L. varieties evaluated in that they exhibited a level of resistance to SF isolates of *Pseudocercosporella*. *H. bulbosum* line S1 was the best of the evaluated lines for incorporating into an eyespot resistance breeding programme.

The wheat breeding lines were in most cases more susceptible to FE isolates than SF isolates, which is consistent with the expected infection of wheat by FE isolates with W-type pathogenicity*. Lines 13, 14 and 28 hold the most promise as a source of eyespot resistance.

*It is of interest that both lines 15 and 16 had VPM parentage, yet only line 16 exhibited low disease severity. This resistance can also only be assumed to have originated from a VPM gene.

8.5 GENERAL DISCUSSION

Following the above considerations it is probably better to only compare results from the Gore trial with growth room trials for evaluating differences between cultivars. Under very severe disease pressure, as demonstrated in the Lincoln trial, all cultivars will show a level of susceptibility.

Bounty had a consistently higher level of resistance than the other wheat cultivars. Other than this cultivar, which contains resistance from Capelle-Desprez, Takahe showed moderate levels of resistance. The barley cultivars were all fairly susceptible to both pathotypes. Of the rye cultivars which were all reasonably resistant, Dominant had the highest level of resistance.

The triticales varied in their response to eyespot. Karere was highly susceptible to both SF and FE isolates and was therefore responding like a wheat cultivar. Lasko however, behaved more like a rye as it exhibited the rye resistance to FE isolates and was susceptible to SF isolates. Salvo and Aranui appear to have the most potential as eyespot-resistant triticales. They exhibited some resistance to both isolate types, and perhaps have to some extent inherited both wheat and rye resistances. With the higher disease pressure in the second growth room trial, all triticale cultivars, except Salvo, retained similar scores. Salvo increased its score as did the rye, barley and most of the wheat cultivars.

The separation of SF and FE inoculum treatments is likely to be marred by the presence of natural infection and spread of inoculum in field trials. This was confirmed by the isolation of SF isolates from FE inoculum treated cultivars (Table 8.7). The comparison of results obtained between the field and growth room trials evaluating the wheat lines, showed that a field technique of this sort is not suitable. The field score for each line was a mean of the sampled tillers which were infected with either fungal pathotype. Rather than determining the mean scores of the plots, the highest scores should be used as the level of susceptibility for each line, as if grown in monoculture the most pathogenic isolates of *Pseudocercosporella* would quickly be selected and increase in number.

Rongotea proved a suitable choice as a susceptible wheat in buffer plots as it scored amongst the most susceptible of the wheat cultivars. Variation in infection of Rongotea buffer plots between the main Lincoln site and the fifth replicate can be explained by the direction of the more frequent southerly wind. The fifth replicate was situated from west to east, and therefore suffered less inoculum spread. It was also situated on the north side of a shelter belt. The main site, being a large block, was more susceptible to inoculum spread by wind and rain splash. Isolates obtained from the Rongotea buffer plots were not always of the same morphology as those applied as treatments to neighbouring plots. The majority of isolates were FE and this suggests that isolates of FE morphology are better able to spread than SF isolates.

9.0 EPIDEMIOLOGY

9.1 STUDY OF INOCULUM BUILD UP AND COMPARATIVE MOVEMENT OF DIFFERENT ISOLATES THROUGH A TRIAL OVER TWO SEASONS.

9.1.1 Materials and methods

Two trial sites were chosen, one autumn-sown at Lincoln and one spring-sown in Southland. Each trial consisted of an area containing three lengths of Rongotea wheat surrounded by a buffer of Omihi oats at Lincoln and Ohau at Gore. As each trial was sown next to a cultivar trial, buffers were effectively two plots wide along one side. The trial site at Lincoln was 44m x 7m, providing 23 plots, 100cm x 90cm in dimension, and in Southland 50.5m x 7m, providing 34 plots. Trial length was dependent upon land availability. Inoculated plots were at the south end of each site so that oncoming wind and rain ensured spread of inoculum along the trial. Plots chosen for inoculation at Lincoln were 2 rows into the trial (i.e. plots 3 and 4) as the end plots emerged unevenly. Colonised oat-grain inoculum was spread evenly, by hand, between rows of plants in the specified plots. Tractor runs for preparatory grubbing and subsequent drilling were made towards inoculated plots in the second season, preventing mechanical spread of inoculum down the trial.

Drilling dates: 28/5/85 and 19/6/86 at Lincoln
26/9/85 in Southland

Inoculation dates: 31/7/85 at Lincoln
28/10/85 in Southland

Inoculum composition: Lincoln

3 FE (K231, K164, K1) and
3 SF (86/2/1, K131, K161) isolates

Southland

3 FE (K211, K165, K1) and
2 SF (K131, K161) isolates

Inoculation rate: 15g m^{-2}

Fertiliser application: Lincoln, 11/9/86
nitrophoska pellets applied using hand-pushed fertiliser spreader at 100 kg/ha

Herbicide application: Lincoln, 8/10/86
10l Hoegrass/Glean mixture for *Phalaris* and broadleaf control applied using hand-held CO_2 sprayer (2.5 l/ha Hoegrass; 20g/ha Glean)

Sampling dates: 16/11/85 and 27-28/1/87 at Lincoln
19/12/85 in Southland

Thirty tillers were collected from the centre row of Rongotea plots and samples numbered beginning with inoculated plots. Samples were scored for percentage tiller infection and isolations from eyespot lesions were made.

9.1.2 Results and discussion

Data describing percentages of infected tillers and morphological types of sampled isolates are given in Table 9.1 and are graphically presented in Fig. 9.1.

Table 9.1 Tiller infection percentages and isolate types in epidemiological trials

	Lincoln site 1985		1986	
Plot no	% infected tillers	isolate types	% infected tillers	isolate types
1	100.00		50.00	3SF
2	100.00	8SF,1FE	48.65	
3	86.66		54.55	2FE
4	73.33		25.00	
5	66.66		52.17	1SF
6	66.66		12.50	
7	43.33		75.00	1FE
8	43.33		75.78	1FE
9	50.00	1FE	21.95	1SF
10	60.00		20.76	5FE,1SF
11	66.66		15.22	
12	63.33		15.00	
13	50.00	3FE	20.00	
14	46.66	1FE	34.78	
15	56.66		69.57	
16	76.66		40.00	
17	66.66		45.61	
18	70.00		26.79	
19	70.00	1FE	7.55	
20	73.33		39.29	
21	73.33		10.64	
22	66.66	2FE	8.70	
23	63.33		4.44	
MEAN	66.66		33.65	
STD DEV.	15.21		22.05	

Gore site

Plot no	% infected tillers (n=30)
1	26.66
2	66.66
3	90.00
4	36.66
5	6.66
6	10.00
7	16.66
8	10.00
9	43.33
10	20.00
11	23.33
12	23.33
13	10.00
14	16.66
15	30.00
16	26.66
17	3.33
18	30.00
19	30.00
20	13.33
21	40.00
22	3.33
23	16.66
24	13.33
25	16.66
26	0.00
27	23.33
28	10.00
29	6.66
30	23.33
31	0.00
32	0.00
33	16.66
34	13.33
MEAN	21.08
STD DEV.	18.58

Fig 9.1 Percentage eyespot-infected tillers in epidemiological trial plots

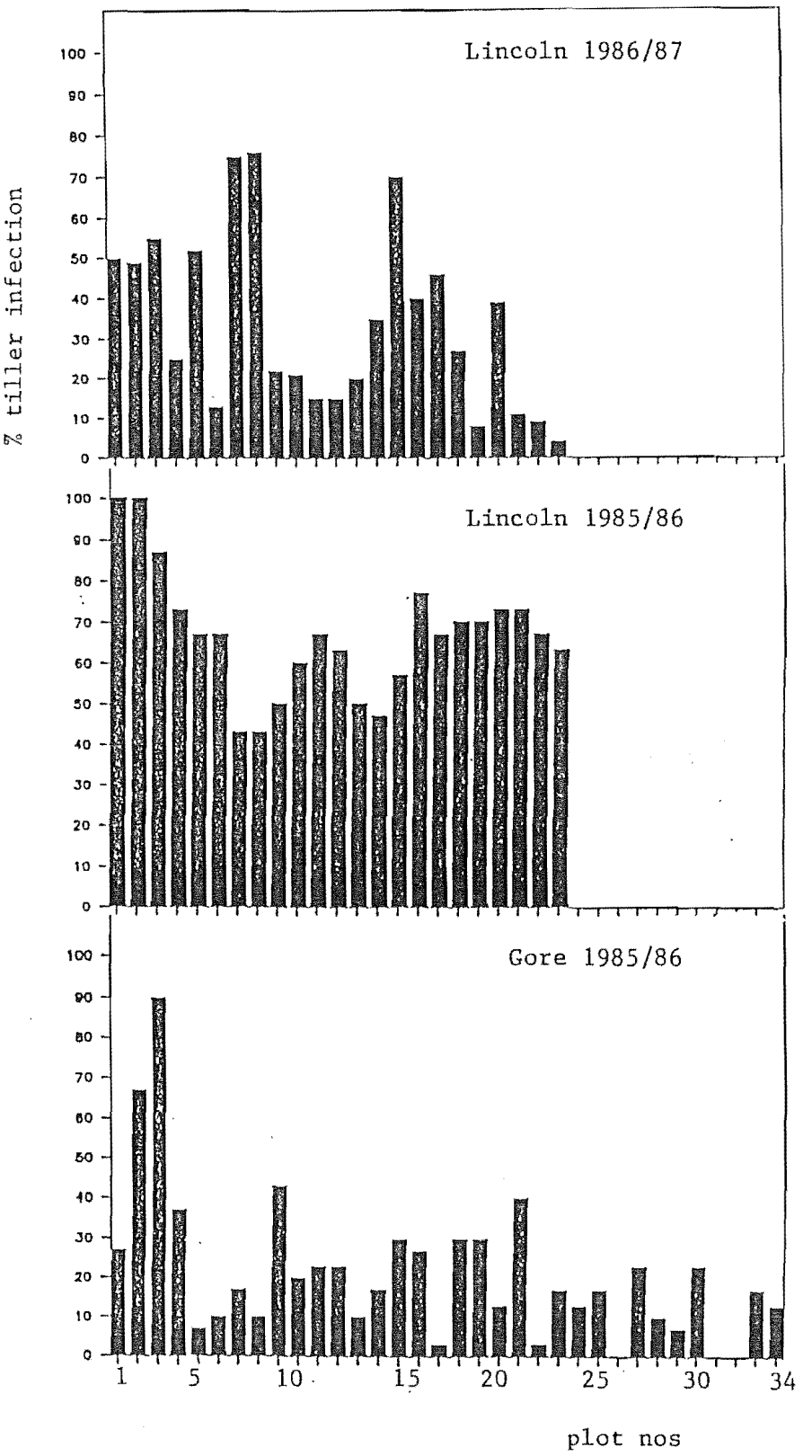


Fig. 9.2 The epidemiological trial site at Lincoln

(a) in NOV. 1985

(b) in JAN. 1987

A

B

After the first season, the Southland trial was mistakenly worked and resown in grass, with the adjacent block left for resowing in wheat, a problem presented by having a distantly-located site. At that site, descriptions were therefore limited to within-season spread. The Lincoln trial was continued over two seasons, though this was disturbed during the second season. Following a combination of roadworking alongside the trial and rain, the trial became waterlogged and seedling emergence and subsequent plant density were therefore low. Take-all infection and *Phalaris minor* Retz were prevalent. The Lincoln trial in each of the two seasons is shown in Fig. 9.2

The same quantities of inoculum were applied to each site although the composition was slightly different. It is possible that virulence of the isolates varied, however pathogenicity tests in Chapter 8 showed isolates K16 4, K1, K16 7 and 85/2/1 to all be virulent. Unless there is a marked difference in aggressiveness of the other isolates, it must be assumed that infection potential was the same at both sites. Neither site had a history of cereal-growing. Scores described only presence/absence of tiller infection and not severity which was actually very low. There was a low percentage of tiller infection in the Gore trial and as severity was not high, isolations were difficult. No *Pseudocercosporella* was obtained, but *Sclerotinia sclerotiorum* (Lib.) de Bary was isolated from many tillers. The percentage of eyespot-infected tillers in the Lincoln trial in 1985/86 was more than twice that occurring in the Gore trial. After the first season, highest levels of infection were in the inoculated plots at the Lincoln site and the plots west of inoculated plots at the Gore site. There was an irregular pattern of infection through uninoculated plots in both trials, although average infection in the Lincoln trial was markedly higher than that in Gore. The level of infection dropped dramatically in the second season.

Isolates obtained from the Lincoln site in the first season demonstrated a higher prevalence of SF morphology from inoculated plots, and a reasonably even distribution of FE morphology across the whole trial. No SF isolates were obtained from uninoculated plots but the sample size was very small. The level of 'natural' inoculum could be estimated as very low or nil as very low levels of eyespot were

found in neighbouring cereal breeders' plots and these levels of infection could have originated from the artificial inoculum from this trial. In the second season of the Lincoln trial, isolates of both morphological types were found across the trial, although FE isolates predominated. SF isolates were collected from plots 1 and 2 to the south of the inoculated plots. These results suggest that FE isolates could spread more quickly and that SF isolates perhaps predominate when in direct competition with FE isolates.

As discussed in Chapter 8, there are major differences between the Southland and Canterbury sites. The time of inoculation at Lincoln would have been too early in the season to mimic natural infection, hence a high level of infection was obtained. Second season inoculum sources were 'natural' and were reflected in the lower level of infection obtained. Rongotea was chosen for this trial as it is a very eyespot-susceptible wheat cultivar, which was confirmed by the cultivar trials. The Lincoln trial was sown from south to north with the southernmost plots being inoculated. In comparison, the Gore trial was sown from southwest to southeast. The oncoming southerly wind may be expected to have moved the inoculum directly down the length of the trial in Lincoln but in Gore the southerly wind would have carried inoculum diagonally across the first few plots and into the neighbouring field. It would have been the less frequent southwesterly and westerly winds which would have carried the inoculum down the trial. The irregular low-level infection through the Gore trial may have arisen from inoculum spread from the inoculated plots or from natural inoculum. The barley trial nearby was also situated in a field with no history of cereals yet transects through the site detected much low-level infection.

From the results of these trials there appears to be a correlation between dispersal of *Pseudocercosporella* and wind and rainsplash.

9.2 INVESTIGATION OF PRESENCE OF PERFECT STATE

In an attempt to detemine the existence of the perfect state of *Pseudocercosporella*, both *in vivo* and *in vitro* methods were employed.

9.2.1 Method

9.2.1.1 *In vivo*

a) Eyespot-infected stubble and alternate hosts were searched in fields of Southland in May, 1986, for structures which may be involved in a sexual stage of the pathogen's lifecycle.

b) A plot of stubble left after harvest in Canterbury was inspected monthly throughout the autumn and winter.

c) Seedlings were inoculated with either; (a) one SF isolate and one FE isolate; (b) two different SF isolates or (c) two different FE isolates.

d) Samples of eyespot-infected stubble were frozen for two months and then observed over a period of six months.

9.2.1.2 *In vitro*

Isolates were subcultured onto WDA, BDA, WEDA and BEDA in case a nutritional or physical requirement was lacking in other media. Plates were kept in both an incubator maintained at 15°C and an incubator maintained at 2°C night/ 15°C day (for 8 months)

9.2.2 Results

No structure found in either the field or laboratory was identified as being associated with the perfect state of *Pseudocercosporella*. Stalked apothecia were prevalent on loose stubble in the chemical spraying trial at the time of spraying, but were not found elsewhere. Following freezing and thawing of cereal straw, sporodochia of *Epicoccum purpurascens* Ehrenb. ex Schlecht formed. The cereal agar induced formation of pseudoparenchyma in contrast to control agar.

9.2.3 Discussion

The recent finding of a *Tapesia* species as the teleomorph of *Pseudocercosporella*, by Dr H. Wallwork in Australia, after leaving infected material for 3 months at 10oC, would suggest its formation in the spring. This could explain why no perfect state was found in the field when searched during autumn and winter. Incubator conditions were also not appropriate.

10.0 EFFECT OF EYESPOT INFECTION ON BARLEY YIELD

10.1 INTRODUCTION

10.1.1 Eyespot-induced yield loss

Most research on the effects of eyespot on yield has been done on wheat. As there are morphological and physiological similarities between wheat and barley, the findings for wheat are likely to give some insight into the effects of infection on barley.

Jorgensen (1964) identified four ways in which the wheat plant is affected by eyespot, although no sharp distinction is possible between the groups: 1) losses from very early infection which kills shoots before elongation occurs; 2) losses from infection before spikelet formation is complete; 3) losses due to necrotic lesions and 4) lodging caused by severe attacks at stem bases.

Estimates of the magnitude of yield loss of wheat as a result of eyespot infection vary. Oort (1936) reported that infected culms yielded approximately 25% less grain than healthy culms, even when not lodged. Straw yield and length were not affected by infection. In the first report of eyespot in New Zealand, Saxby (1943) stated that a slight attack caused no visibly apparent reduction in yield.

Glynne *et al.*, (1945), described grains from eyespot-infected tillers as smaller than healthy ones, with heads producing only half the weight of grain. In severe cases, losses could amount to 60%. Generally, a loss of about 30% in total grain weight was expected. Both grain number per ear and 1000 grain weight were greatly reduced by severe eyespot lesions (Glynne, 1964). Defosse and Rixhon (1968) reported a 2.8% reduction in 1000 grain weight in the presence of light infection by *Pseudocercosporella* and a 12.4% reduction in the presence of severe infection.

Sprague & Fellows (1934) reported a large reduction in culm number in the presence of eyespot infection with heads being smaller with shrivelled grain. There were no differences, however, between germination of grains from healthy and diseased heads.

The yield of cereal plants is made up of many components, hence potential yield reductions which occur as a result of damage to one component at an early growth stage may be compensated for later by other yield components. Scott and Hollins (1978) found that tiller numbers were reduced early in the season, as a result of infection, but this was partially compensated for later. In severely-diseased plots, uninfected tillers produced extra grains and this compensation meant there was no overall effect of eyespot on grain number per ear.

Eyespot-induced yield losses may also vary with cultural and fertiliser treatments (Glynne and Salt, 1958). The percentage of eyespot-infected tillers and plants increased with higher seed rates (Glynne, 1951). Pot experiments showed inoculation decreased yield by 19% in well nourished plants, 86% in stressed plants, 22% in thinly sown plants and 45% in thickly sown plants. Losses were 33% in a series of pot experiments in which all inoculated plants were infected, although there was no general lodging.

Glynne *et al.* (1945) found that plants treated with high nitrogen had an 11% loss of yield owing to eyespot, whereas those treated with low nitrogen had a 23% loss compared with controls. There was much less uniformity in yield among diseased than among control plants.

10.1.2 Lodging as a factor in yield loss.

Glynne (1944) proposed that yield of winter wheat is reduced by *Pseudocercosporella* through both direct effects on the host and indirect effects resulting from lodging. The estimation of direct effects is made difficult in the presence of lodging. A lodged tiller may become colonised by saprophytic fungi, which may also affect yield. Field experimentation with lodging produces results unique to each site. Plants would be more prone to lodging even in the absence of eyespot disease at certain sites, in particular, sites exposed to wind and rain, or containing other fungi such as *Fusarium* spp. Minor infection by *Pseudocercosporella* could be very damaging if this combines with other factors to cause lodging. Effects of *Pseudocercosporella* alone on lodging may only be shown in a controlled environment such as a greenhouse experiment.

A study in which artificial culm breakage was used to simulate the effects of lodging, suggested that early lodging reduces the number of grains per head and later lodging reduces grain size. The percentage of protein from lodged grain was higher, hence quality was higher, than that of grain from standing plants. However, the total amount of protein per hectare was less in lodged areas (Laude and Pauli, 1956). These results only described the restricted capacity of lodged plants to absorb nutrients or synthesise materials. Such losses are not necessarily the same as those ensuing from natural lodging. In addition, losses associated with the mechanical harvest of lodged plants were not considered. Wheat which lodged at ear emergence had the greatest yield loss, and that which lodged before or within one week thereafter regained to some extent an erect position. Further trials with artificially lodged plants have shown a reduction in wheat yield, test weight and kernel weight and have supported the inclusion of straw strength as a factor in wheat breeding programmes (Weibel and Pendleton, 1964).

Scott and Hollins (1978) found a significant linear regression of yield on disease for numerous wheat cultivars, in field trials where plants were grown either through nets to prevent lodging or in unnetted plots to allow lodging. When lodged, susceptible cultivars exhibited a greater yield loss than when standing, however resistant cultivars had similar yield values in either instance. It was concluded that, although dwarf cultivars are very resistant to lodging, severe losses may still occur owing to eyespot infection. Yield losses with lodging and severe eyespot were considered unlikely to exceed 50% and depend upon both degree and timing of disease and lodging.

Yield loss due to eyespot is more closely related to the indirect effects of lodging than to the incidence of severe infection (Scott and Hollins, 1978). 'Disease incidence in spring was not a reliable indicator of the subsequent rate of disease development, or of its effect on yield, but loss in yield sufficient to justify fungicide application was not observed when less than 10% of shoots showed eyespot symptoms in spring.' Difficulties in defining a sampling unit for disease assessment was emphasised. The data did not suggest that

the threshold value should be higher for a resistant cultivar than for a susceptible one, as major differences between cultivars appear established before the earliest assessments were made.

Establishment of disease pressure in yield loss trials.

Jorgensen (1964) found that measurement of eyespot-induced yield loss was difficult both in the establishment of a range of disease severities and in the interpretation of measured severities in terms of disease injury.

Field trials which rely on natural infection must contain disease-free control plots and these must be artificially produced. The use of an effective fungicide to eliminate or at least markedly reduce infection, introduces difficulties as fungicides may enhance plant growth, as with the cytokinin-like action of benzimidazole (Person *et al.*, 1957; Mukhopadhyay and Bandopadhyay, 1977), or be phytotoxic, as with copper-based compounds (Moore *et al.*, 1936). Many fungicides have broad-spectrum activity, so a selective chemical should be chosen. Artificial inoculation of field trials means different levels of disease severity can be produced, from which severity-yield loss relationships may be determined. Unless it is known that a site is free from inoculum, artificial inoculation has the disadvantage of possibly amplifying a wild population. Studies with the take-all fungus, *Gaeumannomyces graminis*, have shown that artificial inoculation tends to produce unnaturally early and rapidly developing epidemics (Jensen & Jorgensen, 1973).

10.1.3 Single shoot yield assessments.

An alternative to field trial evaluations has been developed for estimating disease-induced yield loss. In 1970, Richardson and Rennie estimated wheat yield loss, caused by *Cephalosporium gramineum*, Nis & Ika by marking infected tillers towards the end of a season and later harvesting these along with randomly selected uninfected tillers as controls. Richardson *et al.*, (1975, 1976) assessed effects of various cereal diseases on yield loss by using the same technique. Quantitative yield loss relationships were successfully obtained.

The method had been used to evaluate yellow rust (King, 1976) and take-all (Polley and Clarkson, 1980) and in 1981 it was used for eyespot (Clarkson, 1981). Between 500 and 1000 plants were randomly selected from each test site and all individual shoots were categorised as healthy, slightly, moderately or severely infected with eyespot. Overall yield means were adjusted for the number of plants in each category at each site. Slight eyespot caused no significant reduction in yield however moderate and severe infection resulted in 10 and 36 percent losses respectively. The equation, percentage yield loss (y) = $0.1x^1 + 0.36x^2$, where x^1 is the percentage of shoots with moderate infection and x^2 is the percentage of shoots with severe eyespot, was used to obtain estimates of annual national losses incurred by moderate and severe infection found in ADAS surveys. The mean losses for the years 1975 to 1980 was 0.75% with the range being 0.3-1.2%. The equation previously used, based on work by Glynne (1963) and Scott and Hollins (1974), was $y = 0.5x$ where x is the percentage of severely infected tillers, is less accurate as it does not incorporate moderate infection. Clarkson (1981) discussed disadvantages with the single-shoot assessment method. Lodging could not be examined as it is dependent upon variable crop and weather conditions and the 'severe' category included tissue softened such that lodging should readily occur. Scott and Hollins (1974) reported the ability of healthy shoots to compensate for fewer grains in ears of severely-diseased neighbouring shoots. This cannot be detected in the single-shoot technique. Reduction in tiller numbers (Sprague and Fellowes, 1934; Bojarczuk, 1970; Scott and Hollins, 1974) would not be apparent. Clarkson and Cook (1983) used the technique to study effects of sharp eyespot with the same advantages and disadvantages ensuing.

10.1.4 Aim of experimentation.

Published literature deals extensively with eyespot infection in wheat and little mention is made of its effects on barley. The economic situation may at times favour a swing away from wheat production towards barley and it is common for barley to follow an eyespot-infected wheat crop. 'Eyespot was recently found to be severe in barley crops of South Otago and Southland' (Ballard and Kerse, 1984). Chemical sprays should be applied only if, compared with the

alternative yield loss, they are economically viable, particularly as fungicide resistance often means a fungicide has only a limited potential use.

Experiments were designed to estimate loss of barley yield in the presence of eyespot infection. A comparison of single-shoot yield assessments with a full-scale field trial was also made to assess their suitability for use with eyespot in barley.

10.2 METHODS

10.2.1 Single-shoot yield assessment technique

The single-shoot yield assessment method was utilized for wheat and barley crops. Crops chosen for the study were on the property of R.G. Smith at Waimumu, Southland. One hundred infected and one hundred uninfected tillers were systematically sampled along a transect line. Five transects produced a sample of 1000 tillers. A specified number of control tillers were sampled to decrease error induced from weighting means.

10.2.2 Field trial

10.2.2.1 Plants and fungi

A plot of barley was sown in Southland on 22/10/85 by the staff of the DSIR, Gore. Land restrictions meant this ready-sown barley headland had to be utilised. Advanced lines from which seed is required are used for sowing headlands, hence yield assessments were made of one of these breeding lines, BR302 02. This line had not been bred for eyespot resistance and it seemed reasonable to assume the line would be susceptible. Unfortunately, seed was not available at the time of disease severity assessments, thus eyespot-susceptibility comparisons with named cultivars were not possible. Utilising a pre-sown plot precluded the inclusion of a standard wheat cultivar for comparison.

Oatgrain inoculum was prepared with representative SF and FE isolates bulked up separately. Isolates used were the same as those described in Section 8.2.2. Plants were inoculated on 30/10/85, when at G.S. 12.

The natural inoculum level of the trial site was unknown, hence four different levels of inoculum were applied. The inoculation rate used in the cultivar disease nurseries (Chapter 8) was 15g m^{-2}. Rates chosen for this trial were 10, 20, and 30g m^{-2}. Inoculum was spread evenly by hand between the rows of experimental plants.

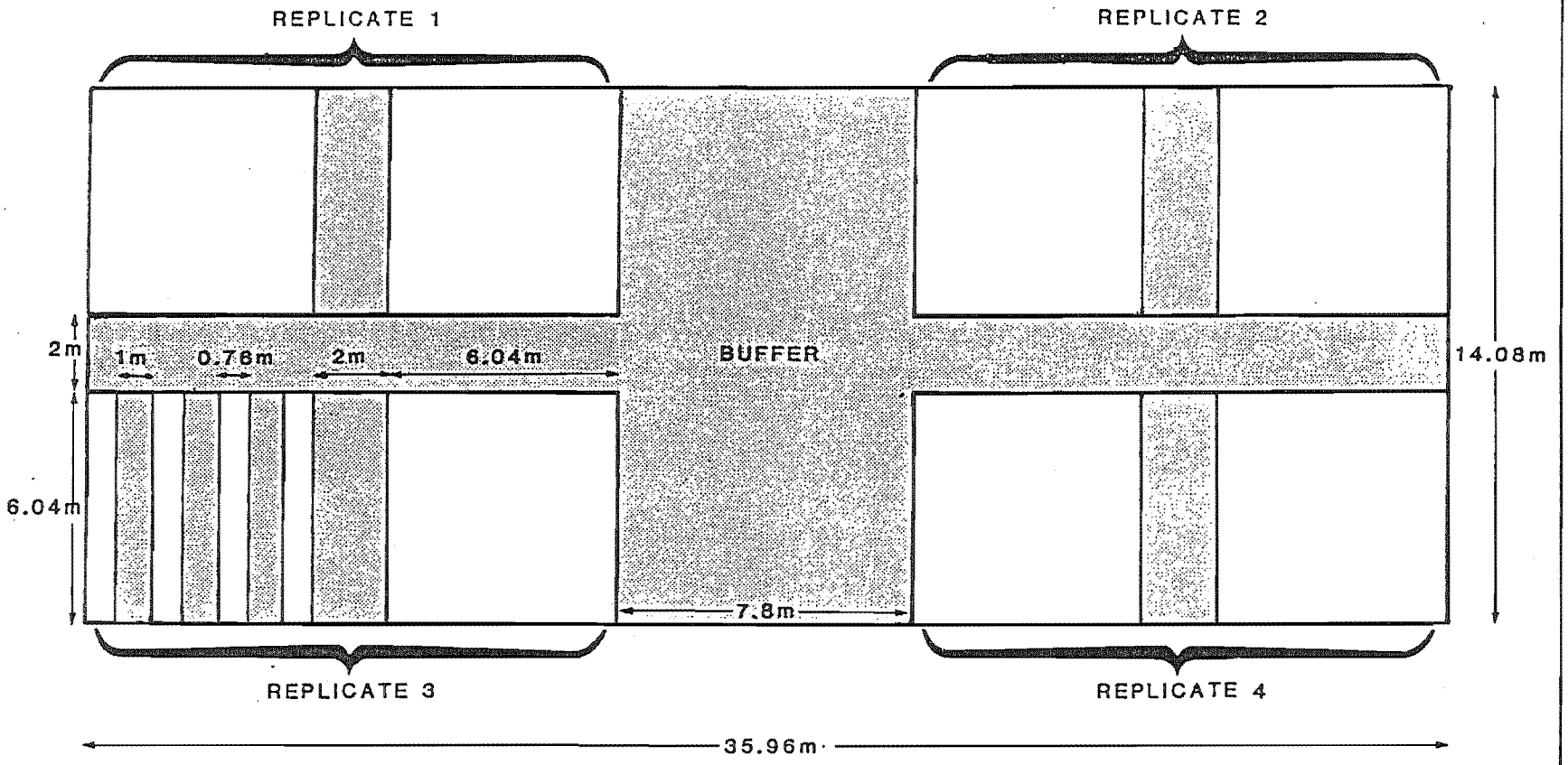
10.2.2.2 Experimental design

A split-plot design was used, with SF and FE isolate growth types as main treatments and inoculation rate as the sub-treatment. The natural eyespot population consists of a mixture of isolate types and in the UK, proportions are known to be seasonally changing in favour of FE isolates (Scott and Hollins 1983, 1984), hence yield relative to inoculum is emphasised as statistically more important than yield relative to isolate type. The trial was situated at the DSIR, Gore, on a south-facing hillside and had no cereal history. Four replications were arranged in a 2x8 array with factors and sub-treatments randomised separately within each, to compensate for variation across the plot. Land uniformity was determined by incorporating covariance. Within each plot, the number of plants was counted to compare emergence across the site. Number of plants per sampled metre within a row was counted at the 1-tiller stage and later in the season tiller numbers reflected variation in plant development.

Figure 10.1 depicts the trial site. Individual plots were 0.76m wide and contained four rows of plants. Only the centre two rows were sampled for harvest determinations to eliminate edge effects. A square block design was used to decrease between-plot variation caused by non-treatment factors. Plot length was designated 6.04m to equal block width, hence the area of each block measured 36.48 m^{-2}. Buffer zones between treatments were 1.00m wide. The buffer zone between blocks was 2.00m in width. The buffer zone between replicates was 7.8m, a width incorporating an irregularly sown area, without separating replicates by a distance greater than necessary.

No herbicides, pesticides or fertilisers were applied to the trial.

Fig. 10.1 Field plan of trial investigating effect of eyespot infection on barley yield



10.2.2.3 Randomisation

Replicate	PLOTS	1 2 3 4	5 6 7 8
	Main treatments/Sub-treatments		
1	SF	C B D A	FE B C A D
2	FE	D B C A	SF D C A B
3	FE	A D B C	SF C B A D
4	SF	B D C A	FE D A C B

A = Inoculum rate 10g m^{-2}
B = Inoculum rate 20g m^{-2}
C = Inoculum rate 30g m^{-2}
D = Control

10.2.2.4 Plant analyses

Sprague and Fellows (1934) reported eyespot appearing later in spring on barley than wheat, with the inner portion of the culms being more readily attacked than the hard outer portion. Less stromatic tissue is formed on barley, less tissue collapse occurs and less culm breakage results.

These symptoms are reflected in the following disease severity scale by which each sample was scored.

0 = no infection

1 = some browning on outer sheaths

2 = much browning on outer sheaths

3 = some browning on inner sheaths

4 = much browning on inner sheaths

5 = completely brown on inner sheath

6 = tissue collapse in lesion area

7 = lodged

Heads were removed with secateurs, total per sample counted and weighed giving mean head weight. The heads were threshed, grain number per sample counted and weighed giving mean grain weight. Preliminary trials showed the seed counter to produce a 0.3% error in counts.

10.2.2.5 Statistical analyses

Analyses of variance, covariance and regression were determined using a GENSTAT computer package. Appropriate data and directive files were created.

Fig. 10.2 Barley yield trial, with stakes marking corners of replicates.

10.3 RESULTS AND DISCUSSION

10.3.1 Single-shoot yield assessment technique.

Preliminary sampling quickly revealed the inadequacies of this technique. All plants were infected in the sampled areas. To determine yield loss because of variation in infection, variable land had to be sampled. Plant density became lower, with tillers spindly as soil compaction increased. These factors alone affect yield significantly. Tiller reduction of infected plants would bias the number of tillers in resulting samples. Selection of 'main' tillers rather than whole plants removes the complication of reduced tillering of infected plants, however, determination of 'main' tillers is subjective as less severely infected tillers would be capable of improved growth. No results were therefore obtained for this technique.

Single-shoot techniques may be more suitable when applied to leaf diseases, such as mildew, which are airborne and sporadic in occurrence within a field. Soilborne diseases may be more uniform in occurrence and severity within a crop, as was apparent in the chosen study crops. Yield determinations require destructive sampling so assessments were of ripe plants.

10.3.2 Field trial

10.3.2.1 Description of site

The barley had suffered no bird damage, shattering or sprouting and the heads were clean. *Rhynchosporium secalis* was prevalent. In the eight weeks prior to harvest, heads had bent over, reducing plant height. This was consistent over the area and was a response to the weight of filling grain. There was no straggling. Tillers were most dense in replicate 1 (Table A6.2). Plots 1-3 of replicate 4 contained rape, radish and chamomile plants. There were no wild grasses. The area of field surrounding the trial was infected with eyespot even though it did not have a history of cereal cropping. Transect lines were taken from the corners of the field through the plots at the centre and visually assessed every 10m. There was no apparent gradient in either presence or severity of eyespot. Fig. 10.2 shows the trial in January.

10.3.2.2 Harvest

On 2/3/86, the number of tillers per metre were counted to determine variation across the site. Four samples per plot were harvested on 3-5/3/86, each consisting of all plants in a metre row. Samples were harvested by hand-pulling plants, including most roots, from the ground. This was the simplest approach, owing to the soil being very damp.

The plant material was too wet to handle and was dried in an oven for 24h prior to being transported to Lincoln for analysis. It was unfortunate that some barley heads were lost during travel preventing measurements of heads per row.

Even minimally-infected tillers had fungal hyphae within the lumens, probably caused by senescence and saprophytic infection.

10.3.2.5 Sample analyses

The trial only demonstrated direct effects of eyespot on barley yield as no lodging occurred. The possibility of land heterogeneity affecting variation was examined using covariate data. Where plant density may have been lower, greater tillering could have occurred in compensation. Scott and Hollins (1974) provided evidence that up to 11% reduction in wheat tiller counts, owing to eyespot infection may occur, however compensation was made later in the season. No significant differences were determined between either seedling emergence measured by plant numbers per metre row, or later plant development, measured by tiller numbers per metre row (Table A5.2). With no significance being obtained with either set of covariate data, the site may be assumed to be uniform with respect to its effects on eyespot development.

Unadjusted ANOVAs of yield components and disease scores are presented in Table 10.1. Mean values for the different variates are listed in Table 10.2.

At the highest inoculum rate, the mean disease score for SF inoculum treated plants was 3.9320 and that for FE inoculum treated plants was

4.3450. Scores were not dissimilar in plants treated with lower rates of inoculum and the lowest rate actually provided the highest mean disease score (4.2750) for SF treatment. The differences in scores were all accounted for by the standard error (0.4730). Time of inoculation and subsequent weather conditions perhaps led to a build up of disease within treated plots to similar levels of medium severity. Highly significant differences ($p=0.001$) were achieved from disease scores between inoculated and uninoculated plots, however mean scores for control plots was 2.1380 within SF treatment blocks and 3.0490 within FE treatment blocks. There was a significant difference ($p=0.016$) between scores of different inoculum types with FE isolates causing higher disease scores. The overall higher scores obtained in plots treated with FE inoculum was reflected in the comparatively higher mean score in the control plots of these blocks, suggesting the source of infection in control plots to have been neighbouring treated plots. Scoring was made difficult by the late stage of plant maturity, however the observed significance between scores suggests the scale was suitable.

Mean head weight was significantly reduced ($p=0.0297$) in treated plots in comparison with uninoculated plots, and decreased from 1.2202g to 1.0902g as the rate of SF inoculum increased and from 1.2282g to 1.1364g as the rate of FE inoculum increased. Control weights were 1.2026g for SF inoculum and 1.2424g for FE inoculum. The mean weight of grain per head between control and treated plots was reduced, but not significantly. Mean weight of grain per head was lowest in plots inoculated with the lowest rate of inoculum, however, this is not unexpected after obtaining the highest disease scores in these plots. Mean grain weights in these plots were 0.969 for SF inoculum treatment and 0.97 for FE treated plots. Control weights for these treatments were 1.1020 and 1.0130 respectively.

There was no reduction in thousand grain weights in treated plots, nor were there significant differences between effects of inoculum types on any of the yield components. The overall mean thousand grain weight was 36.86g.

Total head weight was reduced in inoculated plots, hence either grain weights or numbers could be expected to differ similarly. Differences

between mean weights of grain per head showed a reduction in treated plots, however this was not significant. A decrease in chaff weight could be another explanation, however Oort (1936) reported eyespot infection had no effects on wheat straw yield. Following the results of previous workers, only head yield components were analysed. Aborted tillers could not be accounted for, owing to the loss of head number data.

Regression analyses demonstrated variability in yield values within each individual score category. Variability was similar for both FE and SF isolates. No significant regression equations could be determined.

The trial was sown at the base of a hill where there was poor drainage, hence excess water was always present and even sampled plants had to be dried. Powelson and Rohde (1972) suggested their failure to obtain a yield response in a fungicide trial was a reflection of supplemental irrigation. The plots in the present trial were never moisture-stressed, so the grain still filled in the presence of eyespot infection.

Amounts of eyespot have been shown to vary greatly from year to year, with dry weather delaying development (Scott and Hollins, 1974) with early infection causing a decrease in both head number and thousand grain weight, whereas late infection only caused the latter reduction. Head numbers were not available but assuming trends would be similar in barley as wheat, no decrease would have been expected owing to the low disease pressure and minimal effects on components analysed. The results compare with those of Scott and Hollins in that grain number per ear remained unchanged, rather than those of Glynne (1964) where reductions occurred.

Losses in grain yield have been attributed to severe lesions, and not at all to slight lesions (Ponchet, 1959; Jorgensen, 1964; Defosse, and Rixhon, 1968; Bojarczuk, 1970; Doussinault, 1970; Scott and Hollins, 1974, 1978; Clarkson and Polley, 1981). Infection levels in this barley trial may not have been severe enough to affect all yield components, however a reduction in head weight was clearly shown.

Minor lesions would also contribute to the maintenance of the fungus in stubble as future inoculum.

Table 10.1 Analyses of variance - barley yield trial

		VARIATE:DISEASE SCORE		VARIATE:MEAN WEIGHT PER HEAD	
Source of variation	DF (MV)	MS	F PR	MS	F PR
Block stratum	3	0.8576		0.058741	
Block.fungi stratum					
fungi	1	19.5813	0.016	0.002322	0.814
residual	3	0.7928		0.035257	
Total	4	5.4774		0.027024	
Block.fungi.rate stratum					
rate	3	22.8090	0.001	0.081559	0.027
fungi.rate	3	3.6516	0.131	0.007378	0.790
residual	18	1.7064		0.021112	
Total	24	4.5874		0.026951	
Block.fungi.rate.plant.stratum					
	88(8)	0.9091		0.008347	
Grand total	119				

	VARIATE:MEAN WEIGHT OF GRAIN PER HEAD			VARIATE:THOUSAND GRAIN WEIGHT	
	DF (MV)	MS	F PR	MS	F PR
Block stratum	3	0.04449		20.0047	
Block.fungi stratum					
fungi	1	0.3719	0.399	35.688	0.337
residual	3	0.03868		27.428	
Total	4	0.03831		29.493	
Block.fungi.rate stratum					
rate	3	0.01977	0.591	14.278	0.143
fungi.rate	3	0.01194	0.758	1.062	0.927
residual	18	0.03027		6.962	
Total	24	0.02666		7.139	
Block.fungi.rate.plant stratum					
	88(8)	0.02932		6.706	
Grand total	119				

VARIATE: MEAN NUMBER OF GRAIN PER HEAD

Source of variation	DF (MV)	MS	F PR
Block stratum	3	17.79	
Block.fungi stratum			
fungi	1		0.867 NS
residual	3	22.30	
Total	4	16.91	
Block.fungi.rate stratum			
rate	3	13.01	0.461 NS
fungi.rate	3	10.78	0.540 NS
residual	18	14.48	
Total	24	13.84	
Block.fungi.rate.plant. stratum	88(8)	16.26	
Grand total	119		

VARIATE:SEEDLING NUMBERS

Source of variation	DF (MV)	MS	F PR
Block stratum	3	7.232E 2	
Block.fungi stratum			
fungi	1	1.125E 2	0.644
residual	3	4.285E 2	
Total	4	3.495E 2	
Block.fungi.rate stratum			
rate	3	3.648E 2	0.476
fungi.rate	3	3.002E 2	0.556
residual	18	4.205E 2	
Total	24	3.985E 2	
Block.fungi.rate.plant stratum			
	88(8)		
Grand total	119		

E Y = times 10 to the power of y

TABLE 10.2 Barley yield trial means

RATE	10 g m^{-2}	20 g m^{-2}	30 g m^{-2}	Control	SED (fungi rate)
VARIATE DISEASE SCORE					
INOCULUM TYPE					
SF	4.2750	3.4480	3.9280	2.1380	0.4730
FE	4.3110	4.9610	4.3450	3.0490	
VARIATE MEAN WEIGHT PER HEAD (g)					
INOCULUM TYPE					
SF	1.2202	1.1686	1.0902	1.2026	0.05952
FE	1.2282	1.1548	1.1364	1.2424	
VARIATE MEAN WEIGHT OF GRAIN PER HEAD (g)					
INOCULUM TYPE					
SF	0.9690	1.0190	0.9910	1.1020	0.0565
FE	0.9700	0.9700	0.9830	1.0130	
VARIATE MEAN NUMBER OF GRAIN PER HEAD					
INOCULUM TYPE					
SF	25.95	27.91	26.90	26.85	1.754
FE	26.00	26.71	27.12	27.82	
VARIATE THOUSAND GRAIN WEIGHT (g)					
INOCULUM TYPE					
SF	37.38	36.44	36.83	37.98	1.700
FE	37.30	36.29	36.24	36.39	

11.0 GENERAL DISCUSSION

The present study aimed to describe the N.Z. eyespot population and to determine its variability, particularly in terms of morphology, pathogenicity and fungicide sensitivity.

Early literature described the causal organism, its characteristics, hosts and optimal conditions for growth and development, whilst considering its epidemiology to fit that of a 'simple interest' disease, *sensu* Vanderplank (1963) (Rowe and Powelson, 1973b). Nirenberg (1981, 1984, 1985) recognised the variability of the organism and attempted to classify it into different species and subspecies. Crop rotations and hygiene were considered suitable control measures (Slope and Etheridge, 1970) and the introduction of Capelle-Desprez and VPM1 resistance was a major step forward in preventing eyespot development. The introduction of systemic fungicides facilitated a new form of control and the subsequent development of fungicide resistance provoked further interest.

Historically, eyespot has been considered a disease most important on winter-sown cereals for a combination of reasons including their prolonged exposure to inoculum at early stages of development whilst the crop microclimate is favourable for infection (Dickens, 1964; Bruehl *et al.*, 1968; Hollins and Scott, 1980). Commercial crops in Southland develop more severe disease than those in Canterbury. Winters are cold and wet in Southland, hence crops tend to be spring-sown, in contrast to those autumn-sown in the drier climate of Canterbury. Inoculum, when present, builds up in successive cereal crops and disease levels in second-year and subsequent crops are usually more severe than in first-year crops. To ensure good infection in the present trials, which were at sites not previously sown with cereals, inoculation was undertaken early in the season when plants were at G.S 12-13. During the trial seasons, Southland experienced twice as much rainfall, but similar temperatures to Canterbury. More disease developed in the autumn-sown, winter-inoculated Canterbury trials than in the spring-sown, spring-inoculated Southland trials. The second-season crop of Rongotea wheat at Canterbury had a reduced disease severity, which was of a level more consistent with

naturally-infected crops, however being a second-year crop a reduction in disease severity was unexpected.

It is suggested that the difference in disease severity between the two sites was a result of the inoculation time in Canterbury. Artificial inoculation in Denmark produced unnaturally early and rapidly-developing epidemics (Jensen and Jorgensen, 1973). Glynne *et al.*, (1945) stated that artificial inoculation resulted in all plants becoming infected at the same time, which would be unlikely to occur in the field where some plants nearly always escape infection. Disease severity at Lincoln would have been increased by the prolonged time of exposure to inoculum combined with effects of artificial inoculation, suggesting the major natural infection period to be later than previously anticipated.

Inoculum from soil debris, in the form of vegetative hyphae and conidia, may initiate infections early in the season when environmental conditions are suitable. This would explain the damage incurred in European winter-sown cereals which experience high rainfall.

Eyespot is not severe enough in Canterbury to warrant chemical control. In Southland, chemicals are employed for control of eyespot, but are not sprayed until G.S. 31 which is relatively late in the season for a disease which supposedly has its major infection period early in the season, which suggests that inoculum may still be important in the spring. This is supported by work of Rowe and Powelson (1973b) in East Oregon, an area of similar annual rainfall to Lincoln. They identified two main infection periods, autumn and spring, and during both severe and mild epidemics, peak infection levels developed late in the season as a result of spring infections. Secondary infection was considered unimportant.

The epidemiological trials examined the dispersal and buildup of isolates of the two major pathotypes. Conidia have been described as only capable of dispersing 1-1.5m (Rowe and Powelson, 1973). The finding of a teleomorph of *Pseudocercosporella* in Australia indicates the probability of an alternative inoculum source, ascospores, which

would also introduce new genetic material into the population. Until its occurrence and frequency in nature is determined, its role in the epidemiology of the pathogen will remain unknown. Ascospores from species such as *Mycosphaerella graminicola* are capable of dispersing over long distances but others such as *Pyrenophora graminea* (Died.) Ito & Kurib play only a minor role in disease spread. *Pseudocercosporella* isolates of FE colony morphology and hence W-type pathogenicity appeared to spread more quickly through the Lincoln trial than those of SF morphology and R-type pathogenicity. Isolate sample numbers were low, but the results were also supported by observations made of the cultivar trials. These observations are not consistent with laboratory findings of SF isolates exhibiting more prolific sporulation in culture than FE isolates. The epidemiology trials clearly demonstrated the importance of wind on inoculum dispersal.

Growth room trials demonstrated a stronger pathogenicity of FE isolates than SF on wheat cultivars. The current dominance of R-type isolates in the U.K. population (Scott and Hollins, 1985) has been suggested (Bateman *et al.*, 1986; Hoare *et al.*, 1986) to be related to either the spraying of fungicides or the increased growing of barley. No fungicides had been used at the sites of the present trials, suggesting this not to be a selection pressure. As FE isolates are more pathogenic on wheat than are SF isolates, a wheat host may help compensate for any competitive pressures provided by the presence of SF isolates. Barley, however, is equally susceptible to both pathotypes, hence in an environment such as the U.K. where barley is widely grown, SF isolates may have an overall advantage. In experimental fungicide trials using wheat (Hoare *et al.*, 1986; Bateman *et al.*, 1986) a major change to SF was detected within only three years. This would presumably be related to the artificial inoculum which comprised equal numbers of SF and FE, benomyl-resistant and sensitive isolates from which exponential change probably occurred. In the wild population, these proportions would be different and this may explain why it has taken so much longer for the change to occur.

When grown on agar, a SF isolate was able to inhibit growth of a FE isolate and this could provide an explanation for the change to predominance of SF types noted in the U.K. This could also explain

the higher number of SF isolates obtained in field trial plots inoculated with both types.

Results from electrophoretic studies showed FE isolates, no matter how variable their morphology, produced the same major gel bands for esterase isozymes, whereas all SF isolates of similar variability produced different, consistent major bands. Assuming it is prudent to split the species, these results, combined with the field and agar results would point to the two pathotypes being two separate species.

The N.Z. cultivars and isolates generally interacted as those recorded overseas. N.Z. isolates of FE and SF morphology showed the expected association with W-type and R-type pathogenicities respectively. Bounty, containing Capelle-Desprez resistance, was the most resistant wheat cultivar. The others were all susceptible except for Takahe, which showed moderate levels of resistance. Dominant was the most resistant of the rye cultivars and the triticales responded in varying ways to the different pathotypes. The barley cultivars were highly susceptible to both morphological types of the fungus. In growth room trials with FE isolates, the barley cultivars responded similarly to the moderately-resistant wheat cultivars. They were generally the most susceptible of all species to SF isolates, however disease scores obtained in the yield trial were not consistent with this. Higher scores resulted from inoculation with FE isolates than with SF isolates, although at the lowest inoculum level, scores were similar for both. The scores for each treatment differed, but none were low enough to suggest the breeding line used in the yield trial had a level of resistance to eyespot. Spread of isolates between treatment plots would have obscured the results, however, and the cultivar field trial results were also not as clear as those obtained in growth room trials.

The mean weight per head was significantly reduced in barley plots inoculated with *Pseudocercosporella* compared with uninoculated control plots and this is consistent with previous findings of effects of *Pseudocercosporella* on wheat (e.g. Glynne 1945).

An investigation of chemical control of *Pseudocercosporella* identified two types of resistance responses. Resistance to benomyl was noted in

some 15% of isolates from the Southland population. Resistance levels varied between sites, and some sites contained greater numbers of resistant isolates. Data from the U.K. (King and Griffin, 1985) suggests that the threshold value for control failure to be 30% resistance. Overall resistance was higher in the second of three surveys, however, the sample size in that year was too small to be truly indicative. In N.Z., growers maintain short crop rotations with few sites having more than three years of cereals, and sprays being rarely used in the first season. These practices appear to be containing levels of resistance and it is hoped that by continuing careful management programmes, resistance will not become the problem it has in the U.K.

A second type of resistance response, a low-level resistance or insensitivity, was identified in *Pseudocercosporella*, to the demethylation-inhibiting chemical, prochloraz. The mode of action of members of the DMIs is apparently similar, yet they provoke different responses in different species of fungi. Genetic studies with the apple scab fungus, *Venturia inaequalis*, have shown insensitivity to be under nuclear gene control, exhibiting complete resistance (Stanis and Jones, 1985). Responses of *Pseudocercosporella* to prochloraz are numerous, inciting small changes in EC50 values at concentrations similar to the rates applied commercially. The responses are not typical of those incited by nuclear genes, but may be related to gene regulation or amplification. It is possible that there is some form of extrachromosomal control. There is an increasing awareness of the importance of cytoplasmic genes, frequently located in dsRNA, in fungi (Van Alfen *et al.*, 1975). It is possible that viruses could also play a role. If extrachromosomal fungicide-insensitivity gene/s are present in *Pseudocercosporella*, there is a potential for their use in furthering our understanding of the extranuclear control in fungi, as they would provide ideal markers in genetic studies. It is possible that this type of inheritance occurs in other fungal species.

The field trial investigating chemical control of eyespot indicated the use of chemical mixtures as an improved strategy. A mixture of benomyl and prochloraz, each at half rates, should both maintain disease control and prevent development of fungicide resistance,

whilst remaining cost-effective. Such a strategy must also be considered in conjunction with integrated control practices.

NOTE

Further to the completion of this work, the perfect state has been named *Tapesia yallundae* (Wallwork, *pers. comm.*) and has been found on standing stubble at Riverton in Southland (Sanderson, *pers. comm.*). This is the first finding *in vivo* and was at a site near the coast, hence subjected to more temperate conditions than sites inland. It appears that this species only represents fast-even isolates and that stalked apothecia previously found on stubble in the chemical spraying trial of the present study represent the slow-feathery perfect state. These apothecia are similar to those of *Cyathicula* and it is therefore likely that the two pathotypes should be designated to different genera. This would substantiate the indications obtained from the morphological and isozyme analyses.

Scott and Hollins (1987) reported substantial growth of some *Pseudocercosporella* isolates on 0.5 ug ml^{-1} prochloraz. Some variation in growth was associated with different stock cultures of the same isolate and EC50 values were higher when tests were assessed after three weeks rather than two.

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APPENDICES ERRATUM P 262 should appear between P 259 and P 260

LIST OF CONTENTS

APPENDIX 1 - SURVEY SITES
TABLE A1.1
1983/1984 SOUTHLAND SURVEY SITES

PADDOCK NO.	LOCATION	CROP	/	CULTIVAR	HISTORY	LODGING	NO. EYESPOT-INFECTED TILLERS	
1	Waianiwa	Wheat	/	Kopara	-	-	25/46	(54.35%)
2	Waianiwa	Barley	/	-	-	-	97/100	(97.05%)
3	Waianiwa	Barley	/	-	-	-	71/108	(65.74%)
4	-	Wheat	/	Oroua	-	-	25/64	(39.03%)
5	Waianiwa	Barley	/	-	-	20%	39/129	(30.23%)
6	-	Wheat	/	Takahe	-	-	17/45	(37.70%)
7(a)	Otahuti	Barley	/	-	-	-	10/100	(10.00%)
7(b)	Otahuti	Barley	/	-	2wh +1 ben	-	100/100	(100.0%)
8	Drummond	Barley	/	-	-	-	45/125	(36.00%)
9	Drummond	Wheat	/	Rongotea	-	-	17/89	(19.10%)
10	Drummond	Wheat	/	Takahe	-	-	0	(0.00%)
11	Drummond	Barley	/	-	-	-	85/100	(85.00%)
12	Heddon Bush	Wheat	/	Takahe(?)	-	-	92/100	(92.00%)
13	Heddon Bush	Barley	/	-	-	-	100/100	(100.00%)
14	Heddon Bush	Barley	/	-	-	-	100/100	(100.00%)
15	Heddon Bush	Barley	/	-	-	-	100/100	(100.00%)
16	Heddon Bush	Barley	/	-	-	-	100/100	(100.00%)
17	Heddon Bush	Wheat	/	-	-	-	5/76	(6.58%)
18	Heddon Bush	Wheat	/	Kopara	-	-	17/56	(30.36%)
19	Heddon Bush	Wheat	/	Takahe	-	-	18/93	(19.36%)
21	S. Timaru	Oats	/	-	-	lodging	95/100	(95.00%)
22	S. Balclutha	Barley	/	-	-	-	0	(0%)
23	S. Edendale-Dacre	Barley	/	-	-	0	-	
24	Balfour	Barley	/	-	-	0	-	
25	N. Invercargill	Wheat	/	-	-	0	-	
26	N. Invercargill	Barley	/	-	-	yes	-	
27	Gardyne	Wheat	/	Kopara	-	-	50/67	(74.63%)
28	Gardyne	Wheat	/	Mixed	-	0	44/53	(83.02%)
29	Gardyne	Wheat	/	-	-	0	100/100	(100.00%)
30	Gardyne	Barley	/	-	-	0	-	
31	Waikaka Stream	Wheat	/	-	-	lodged, re-elongated	72/80	(90.00%)
32	S. Otama	Wheat	/	-	-	0	100/100	(100.00%)
32.5	Waikaka Stream	Barley	/	-	-	0	100/100	(100.00%)
33	Otama-Kelso	Wheat	/	-	-	0	17/81	(20.99%)
34	Kelso	Wheat	/	-	-	-	59/60	(98.33%)
35	Kelso	Wheat	/	-	-	lodging	54/57	(94.74%)
36	Tapanui	Wheat	/	-	1st year cereal	0	55/56	(98.21%)
37	W. Pukerau	Rye	/	-	-	0	84/129	(65.12%)
38	Pukerau	Barley	/	-	-	-	70/76	(92.11%)
39	Pukerau	Barley	/	-	-	0	25/56	(41.18%)
40	Pukerau	Oats	/	-	-	-	1/85	(1.18%)
41	Conical Hill	Wheat	/	-	-	lodged, reelongated	48/69	(69.57%)
42	-	Wheat	/	Takahe(?)	-	-	-	
43	-	Barley	/	-	-	-	-	
44	Conical Hill	Barley	/	mixed	-	-	-	
45	Waitane	Wheat	/	Tiritea	-	0	100/100	(100.00%)
46	Waitane	Wheat	/	Tiritea	-	-	100/100	(100.00%)
47	Waitane	Wheat	/	Tiritea	-	0	64/70	(91.43%)
48	Te Tipua	Barley	/	-	-	lodging, N2	100/100	(100.00%)
49	Waitane	Wheat	/	-	-	-	84/88	(100.00%)
50	Winton	Barley	/	-	-	-	100/100	(100.00%)
51	Winton	Barley	/	-	2wh., 2ben.	-	100/100	(100.00%)
52	Winton	Barley	/	-	2wh., 2ben.	-	100/100	(100.00%)
53	Winton	Barley	/	-	oats, 3-5wh., ben.	0	100/100	(100.00%)
54	Winton	Wheat	/	-	-	-	100/100	(100.00%)
55	Winton	Wheat	/	Takahe	-	-	100/100	(100.00%)

56	Winton	Wheat	/	-	-	-	100/100	(100.00%)
57	Winton	Wheat	/	Karamu	-	lodging, N2	43/71	(60.56%)
58	Winton	Wheat	/	Rongotea	-	-	94/113	(83.19%)
59	Winton	Wheat	/	-	5wh., 5ben.	-	69/92	(75.00%)
60	N. Winton	Barley	/	-	-	0	85/90	(94.40%)
61	Limehill	Wheat	/	-	-	0	100/100	(100.00%)
62	Limehill	Wheat	/	Takahe	2nd yr	-	53/78	(67.95%)
63	Centre Bush	Barley	/	Mixed	-	0	100/100	(100.00%)
64	Fernhill	Barley	/	Mixed	-	0	85/89	(95.51%)
65	Benmore	Wheat	/	-	-	-	72/74	(97.30%)
66	Benmore	Barley	/	-	-	-	100/100	(100.00%)

TABLE A1.2
1984/85 SOUTHLAND SURVEY SITES

SITE	SAMPLE	LOCATION	CROP / CULTIVAR	HISTORY *	LODGING	NO. EYESPOT-INFECTED TILLERS
1		Gore, DSIR Septoria nursery	Wheat / -	no benomyl	some	100%
2		Moneymore, Clark	Barley / Koru	4th yr cereal/no benomyl	much	severe
3a		Gore, Temple	Triticale / -	no benomyl	-	not above 2nd node
b	1	Gore, Temple	Wheat / Takahe	4th benomyl+CCC	-	severe
c	2	Gore, Temple	Barley / Triumph	4th benomyl	yes	100%
d	3	Gore, Temple	Wheat / -	4th cereal	-	100%
e		Gore, Temple	Triticale / Lasko	4th benomyl	straggling	-
4		N. Gore, Brock	Wheat / Takahe	1st cereal, no benomyl	-	-
5		Winton, P. King	Barley / Kym	2nd cereal, no benomyl	some	100% minor
6	10	Hedgehope, M.Evans	Barley / Triumph	3rd cereal, no benomyl	none	-
7		Mataura, P. Brunstan	Barley / Golden Promise	1st cereal, no benomyl	yes	100%
8a		Mataura, H.C. Copeland	Barley / Triumph	3rd cereal, benomyl	none	100%
b	4	Mataura, H.C. Copeland	Barley / Golden Promise	1/4 3 benomyl	yes	-
9a	9	Hedgehope, Wason	Wheat / Takahe	3rd benomyl, BayletonBM 2x	-	100%
b		Hedgehope, Wason	Wheat / Takahe	5th benomyl, BayletonBM 2x	-	100%
c		Hedgehope, Wason	Barley / Kym	12th benomyl	none	-
10a	5	Chatton, Gardyne	Wheat / Tiritea/mixed	1st cereal, no benomyl	-	45.83%
11b	6	Chatton, Gardyne	Barley / Goldspear	5th cereal, 2 benomyl	some	100%
12c		Chatton, Gardyne	Wheat / Takahe	2//2 4 benomyl	-	100%
13d		Chatton, Gardyne	Wheat / Takahe	2///12 benomyl	none	100%
14		Clydevale, C. Shore	Barley / Goldmarker	4th benomyl	yes	-
15		-	Wheat / Tiritea	2nd cereal, no benomyl	-	100%
16	11	Clydevale, E. Young	Wheat / -	-	-	minimal
17	12	Clydevale, C. Shore	Barley / Triumph	-	-	-
18	7	Wairuna	Barley / Golden Promise	4th cereal	-	100%
19	13	Wharatoa Rd	Barley / Golden Promise	4th cereal	-	100%
20		Clydevale, C. Shore	Wheat / Oroua	-	-	-
21		Gore, DSIR	Triticale / -	- , lesions above 4th node	yes	severe
22		N. Gore, Brock	Wheat / Takahe/Tiritea	-	-	100%
23		Moneymore, Clark	- / -	- , lesions above 3rd node	-	100%
24a		Flints Bush, O. Fallow	Barley / Triumph	11th + benomyl	-	100%
24b		Flints Bush, O. Fallow	Barley / -	1st year cereal	-	-
24c	8	Flints Bush, O. Fallow	Wheat / Rongotea	5th + benomyl	-	-
24d		Flints Bush, O. Fallow	Oats / -	2/3	-	sharp eyespot only
24e		Flints Bush, O. Fallow	Wheat / Rongotea	-	-	-
24f		Flints Bush, O. Fallow	Wheat / -	2/3	-	100%

KEY

* x/y = x years cereals followed by a non-cereal crop followed by y years cereals

// = 2 years non-cereal crops

CCC = chlormequat

47	Dacre, M. Black	Triticale / Lasko	-	benomyl	no sample	-
48	W. Gore, A. Hanson	Barley / Golden Promise	3rd/1	benomyl	-	56/336 (16.66%)
49	W. Gore, A. Hanson	Barley / Golden Promise	-		-	-
50	Mataura, D. Falconer	Wheat / Tiritea	2nd	benomyl	-	24/52 (46/15%)
51	Mataura, D. Falconer	Barley / Triumph	2nd	benomyl	-	1/130 (0.77%)
52	Mataura, D. Falconer	Wheat / Oroua	1st/1/3 benomyl,prochloraz		-	a)4/99 (4.04%) b)1/100 (100%) Urea trial c)9/31 (29.03%) d)12/183 (7.34%)
53	Mataura, D. Falconer	Barley / Triumph	3rd/2		-	13/113 (11.5%)
54	Waitane, Nind bros	Wheat / Oroua	1st	prochloraz	-	65/212 (30.66%)
55	Waitane, Nind bros	Barley / Kym	1st		-	16/100 (16%)
56	W. Gore, R. Scott	Ryegrass / ?	2nd		100%	48/188 (25.53%)
57	Waitane, K. Miller	Barley / Fleet	1st		-	38/164 (eyespot) 93/164 (sharp eyespot)
58	Waitane, ?	Barley / -	-		-	-
59	Waitane, ?	Barley / -	-		-	110/278 (39.57%)
60	Tuturau, J. Dickie	Barley / Fleet	-		none	-
61	Tuturau, J. Matheson	Barley / -	-		-	9/225 (4.00%)
62	Tuturau, J. Matheson	Barley / -	-		-	-
63	Tuturau, ?	Barley / -	-		-	46/376 (12.23%)
64	Tuturau, ?	Barley / -	-		-	6/47 (12.77%)
65	Tuturau, Campbell	Wheat / -	-		-	-
66	W. Gore, K. Bowmar	Barley / Koru	-	no, but did later after rain		34/110 (10.97%)
67	Willowbank, P. Verkerk	Barley / Goldmarker	4th	1/10 benomyl, prochloraz	-	29/270 (10.74%)
68	Willowbank, P. Verkerk	Wheat / Oroua	1st		-	26/274 (9.49%)
69	Willowbank, P. Verkerk	Barley / Fleet	2nd/2	1/10 benomyl prochloraz	-	24/199 (12.06%)
70	Willowbank, P. Verkerk	Barley / Triumph	8th, prochloraz 1/10benomyl///benomyl		-	20/298 (6.71%)
71	W. Gore, J. Allan	Barley / Triumph	7th//4	benomyl	-	5/22 (22.73%)
72	W. Gore, J. Allan	DSIR trial				
73	W. Gore, J. Allan	Wheat / Oroua	no cereals for 30yrs		-	-
74	W. Gore, Winsloe	Barley / -	-		-	31/276 (11.23%)
75	Invercargill, E.B. Fallow	Barley / Triumph	ref. 1984/5		-	31/68 (45.59%)
76	Kelso, B.R. Leitz	Barley / Hassan	5th		-	100/146 (68.49%)
77	Kelso, B.R. Leitz	Wheat / Crossbow	5th		10%	100%
78	Kelso, B.R. Leitz	Wheat / Abele	6th		-	100%
79	Browns, Zwies	Wheat / Oroua	1st		none	11/94 (11.7%)
80	Browns, Zwies	Wheat / Oroua	2nd		straggling	68/72 (94.4%)
81	Young	Barley / Triumph	8th		-	100%
82	Young (bro.)	Barley / Triumph	2nd (prochloraz)		where N2 sprayed	37/71 (52.11%)
83	Nth Tapanui, Guise	Barley / Triumph	2nd		-	3/25 (12.00%)
84	Kelso, I. Logan	Barley / Triumph	1st		some	38/48 (79.16%)
85	Kelso, R. Chittock	Wheat / Oroua	benomyl (prochloraz)		-	-
86	Kelso, S.K.Scarlet	Wheat / Oroua	3rd		-	100%
87	Kelso, S.K. Scarlet	Wheat / Oroua	2nd/1		some straggling	67/77 (87.01%)
88	Paterson	Wheat / Oroua	1st		-	37/138 (26.81%)
89	Willowbank, G. Morrison	Barley / Goldmarker	8th, prochloraz		-	100% (both halves)

CENTRAL OTAGO

1.	Lauder,Omakau,M. Williams	Rye / Rahu	1st		none	-
2	Lauder,Omakau,M. Williams	Wheat / Oroua	1st		-	-
3	Lauder,Omakau,M. Williams	Wheat / Oroua	4th		-	-
4	Nth Omakau,L. Shore	Rye / -	3rd		sheepgrazed	14/187 (16.09%)
5	Nth Omakau,L. Shore	Rye / -	1st		-	6/42 (14.29%)
7	Gimmerburn	Rye / -	-		-	6/23 (26.09%)

SOUTH CANTERBURY

1	R. Sounness	Wheat / Bounty	4th, benomyl	-	-
2	R. Sounness	Wheat / Oroua	-	-	79/148 (53.38%)
3	P. Wilson	Barley / Triumph	-	-	2/120 (53.38%)
4	P. Wilson	Wheat / Oroua	1st	none	-
5	D. Stewart	Wheat / -	-	-	38/272 (13.97%)
6	Russell	Barley / Magnum	2nd	none	7/30 (23.30%)
7	P. Campbell	Barley / Kym	2nd	none	-
8	P. Campbell	Barley / -	-	-	-
9	P. Wilson	Barley / Triumph	3rd	-	4/152 (2.63%)

NORTH CANTERBURY

1	?	Barley / -	1st	none	18/41 (43.90%)
2	?	Barley / -	1st	none	6/65 (9.23%)
3	?	Barley / Triumph	2nd	none	32/101 (31.68%)
4	?	Wheat / -	-	-	7/63 (11.11%)
5	?	Barley / -	-	none	29/94 (30.85%)
6	?	Wheat / -	-	none	23/88 (26.14%)
7	?	Barley / -	-	100% patchy	11/93 (11.83%)
8	?	Barley / -	-	some	10/115 (8.7%)
9	?	Barley / -	-	some	-
10	?	Barley / -	-	none	19/110 (17.27%)
11	N. Dalley	Rye / -	3rd	-	-
12	Taggart	Barley / Goldspear	2nd	some, as dense	10/153 (6.54%)
13	Taggart	Wheat / Rongotea	2nd	none	15/71 (21.13%)
14	Taggart	Wheat / Rongotea	2nd	none	6/85 (7.06%)
15	Taggart	Barley / Rongotea	2nd	-	9/64 (14.06%)
16	Taggart	Wheat / Advantage	2nd	some	27/57 (47.37%)
17	Gardener	Wheat / Karamu	1st	none	13/94 (13.83%)
18	?	Wheat / -	1st	-	-

TABLE A1.3
1985/86 NEW ZEALAND TRIAL SITES

PADDOCK NO.	FARMER	CROP / CULTIVAR	HISTORY	LODGING	%EYESPOT-INFECTED TILLERS
1	N. Gore, D. Temple	Barley / Triumph	5th benomyl	none	10/158 (6.33%)
2	N. Gore, D. Temple	Barley / Triumph	5th benomyl	none	18/172 (10.47%)
3	N. Gore, D. Temple	Barley / Fleet	5th benomyl	none	29/177 (16.38%)
4	N. Gore, D. Temple	Barley / Fleet	6th benomyl	none	11/288 (3.82%)
5	N. Gore, D. Temple	Wheat / Oroua	4th benomyl	none	27/175 (15/43%)
6	Chatton, C. Gardyne	Wheat / Tiritea	4th benomyl	none	198/308 (62.66%)
7	Chatton, C. Gardyne	Barley / Golden Promise	2nd/4 prochloraz (benomyl)	-	9/262 (3.44%)
8	Chatton, C. Gardyne	Wheat / Oroua	benomyl	-	-
9	Chatton, C. Gardyne	Wheat / Tiritea	6th/3 benomyl	-	48/221 (21.72%)
10	Chatton, C. Gardyne	Wheat / Oroua	1st Cycocel	-	2/216 (0.93%)
11	Chatton, C. Gardyne	Wheat / Tiritea	1st benomyl	-	-
12	W. Invercargill, O. Fallow	Barley / Golden	15+ benomyl (prochloraz/benomyl)		5/246 (2.03%)
13	W. Invercargill, O. Fallow	Barley / Fleet	3rd prochloraz	none	-
14	W. Invercargill, O. Fallow	Barley / Fleet	3rd benomyl	none	-
15	W. Invercargill, O. Fallow	Wheat / Oroua	1st/4	-	43/249 (17.27%)
16	W. Invercargill, O. Fallow	Barley / Triumph	1st/3 benomyl	-	23/114 (20.68%)
17	W. Invercargill, O. Fallow	Barley / Golden Promise	4th/5 prochloraz	-	25/172 (14.54%)
18	Moneymore, N. Clark	Barley / Fleet or Goldmarker	6th benomyl	-	48/306 (15.69%)
19	Moneymore, N. Clark	Barley / Fleet or Goldmarker	4th? benomyl	-	7/265 (2.64%)
20	Hedgehope, R. Wason	Barley / Gwylan	2nd	-	-
21	Hedgehope, R. Wason	Wheat / Oroua	1st/?	-	11/216 (5.09%)
22	Hedgehope, R. Wason	Wheat / Oroua	-	none	<1/100 (<1.0%)
23	Hedgehope, R. Wason	Wheat / Oroua	-	none	13/87 (14.94%)
24	Hedgehope, M. Evans	Barley / Koru	5th	-	57/248 (22.98%)
25	Hedgehope, M. Evans	Barley / Koru	6th	-	8/100 (8.00%)
26	Hedgehope, M. Evans	Barley / -	2nd	-	50/328 (15.24%)
27	Waimumu, H. Copeland	Barley / Koru	4th benomyl	-	20/189 (10.50%)
27	Waimumu, H. Copeland	Barley / Goldmarker	2nd/4th benomyl	-	20/189 (10.58%)
28	Waimumu, H. Copeland	Barley / Goldmarker	2nd/4 benomyl	-	5/100 (5.00%)
29	Waimumu, H. Copeland	Wheat / Tiritea	1st	-	13/100 (13.00%)
30	Waimumu, H. Copeland	Wheat / Takahe	2nd benomyl	-	12/100 (12.00%)
31	W. Invercargill, L. Fraser	Barley / Fleet	9th benomyl, prochloraz	-	20/206 (9.71%)
32	W. Invercargill, L. Fraser	Triticale / Wembley	5th benomyl, Cycocel		81/109 (74.31%)
33	W. Invercargill, L. Fraser	Barley+Triticale Triumph	2nd benomyl		Barley, 8/99(8.08%); Triticale, 39/69(56.52%)
34	W. Gore, R.G. Smith	Barley / Triumph	3rd benomyl	-	-
35	W. Gore, R.G. Smith	Barley / Triumph	-	-	14/190 (7.14%)
36	Blandonville, M. Miller	Wheat / Advantage+trial	1st		25/51 (trial); 27/34 (Advant.)
37	Pukerau, P. Pullar	Wheat / Oroua	2nd benomyl, prochloraz	none	12/100 (12.00%)
38	Pukerau, P. Pullar	Barley / Triumph	8th benomyl, prochloraz	none	20/206 (9.71%)
39	Pukerau, P. Pullar	Barley / Triumph	6th benomyl	-	18/259 (69.49%)
40	Pukerau, P. Pullar	Barley / Triumph	4th benomyl	none	5/100 (5.00%)
41	Dacre, M. Black	Triticale / Lasko	4th benomyl	-	179/284 (63.03%)
42	Dacre, M. Black	Barley / Fleet	10th benomyl	-	2/350 (0.005%)
43	Dacre, M. Black	Barley / Triumph	12th benomyl	-	5/100 (5.00%)
44	Dacre, M. Black	Wheat / Oroua	2nd benomyl	-	27/230 (11.74%)
45	Dacre, M. Black	Wheat / Triumph	- benomyl	-	4/71 (5.63%)
46	Dacre, M. Black	Wheat / -	1st	-	15/100 (15.00%)

APPENDIX 2 - DESCRIPTIONS OF ISOLATES
TABLE A2.1

Isolates collected in 1983/84 - This data is base of subsequent surveys

ISOLATE	CROP	2 BENOMYL SENSITIVE(S) RESISTANT(R)	2 PROCHLORAZ SENSITIVE(S)	HYPHAL DIAMETER(cm) after 18 days	ISOLATE DESCRIPTION	GROWTH TYPE
1/1	Wheat	S	-	-	-	FE
1/2	Wheat	S	-	2.20	smooth edge, white ring, high brown lump	FE
1/4	Wheat	S	-	-	-	-
1/6	Wheat	S	-	3.00	smooth edge, white-grey, low, brown lump	FE
1/7	Wheat	S	-	2.55	same as 1/2 but smaller lump, smaller ring	FE
1/9	Wheat	S	-	2.75	similar 46/5, very yellow, white ring	FE
7b/1	Barley	R	-	2.75	same as 1/7	FE
7b/2	Barley	R	-	1.65	slow-feathery, yellow feathers	SF
7b/4	Barley	R	-	2.70	even edges, fluffy colony, green ring, wide grey lump	FE
7b/6	Barley	R	-	3.30	same as 7b/4, 1/1	FE
7b/7	Barley	R	-	2.75	same as 1/7	FE
9/2	Wheat	S	-	-	-	-
11	Barley	S	-	-	-	-
12	Wheat	S	-	-	-	-
14/3	Barley	S	-	2.65	flat lump, grey, white ring	FE
15/2.1	Barley	S	-	2.90	high lump, white, grey, fawn ring	FE
15/2.2	Barley	S	-	2.80	similar but no identical to 15/2.1	FE
15/3	Barley	S	-	2.60	similar to 23/1	FE
15/5	Barley	S	-	-	-	-
15/5	Barley	S	-	-	-	-
16/1	Barley	S	-	-	-	-
16/4	Barley	S	-	-	-	-
16/5	Barley	S	-	-	-	-
23/1	Barley	S	-	2.45	similar to 1/7	FE
23/4	Barley	S	-	0.70	even edge and lump	SF
23/5	Barley	S	-	2.50	white, rust rings	FE
23/7	Barley	S	-	-	-	-
23/9	Barley	S	-	-	-	-
23/10	Barley	S	-	-	-	-
23	Barley	S	-	-	-	-
24/1	Barley	S	-	2.50	large, white lump, yellow/white ring, smooth	FE
25	Wheat	S	-	0.90	slow-feathery, fluffy, dark grey	SF
25/1	Wheat	S	-	-	-	-
26/1	Barley	S	-	-	-	-
26/4	Barley	S	-	-	similar to 14/3	FE
27	Wheat	R	S	-	-	-
30/1	Barley	S	-	-	-	-
30/2	Barley	S	-	-	-	-
30/3	Barley	S	-	-	-	-
30/5	Barley	S	-	-	-	-
30/6	Barley	S	-	-	-	-
31/1	Wheat	S	-	-	-	-
32/2	Wheat	S	-	-	-	-
32/4	Wheat	S	-	-	-	-
32.5/8	Barley	S	-	-	-	-
32.5/10	Barley	S	-	-	-	-

33/1	Wheat	S	-	-	-	-
34	Wheat	S	-	-	-	-
35/1	Wheat	R	-	-	-	-
37/2	Rye	S	-	-	-	-
37/3	Rye	S	-	-	-	-
45/1	Wheat	R	-	2.65	similar to 1/7	-
45/2	Wheat	S	-	-	-	-
45/4	Wheat	S	-	-	-	-
45/6	Wheat	S	-	-	-	-
45/7	Wheat	S	-	2.7	wide, low lump	FE
45/9	Wheat	S	-	3.15	similar to 1/7	FE
46/1	Wheat	R	-	2.85	similar 45/1	FE
46/3	Wheat	S	-	-	-	-
46/4	Wheat	S	-	-	-	-
46/5	Wheat	S	-	2.60	brown, grey lump, white ring, different to others	-
47/1	Wheat	R	-	2.60	similar 7b/6, low, fluffy lump, very dark underneath	FE
47/2	Wheat	S	-	2.65	similar 1/7	FE
47/3	Wheat	S	-	3.10	similar 15/2.2	FE
47/4	Wheat	S	-	-	-	-
47/5	Wheat	S	S	2.50	similar 47/2, lump half black, half white	FE
47/8	Wheat	R	-	2.65	similar 1/7, but brown lump	FE
48	Barley	S	-	-	-	-
49	Wheat	S	S	-	-	-
50/1	Barley	S	-	2.65	similar 1/7	FE
50/5	Barley	S	-	2.55	mottled lump, pale ring	-
50/6	Barley	R	S	2.80	similar 47/3	FE
50/8	Barley	R	S	3.00	similar 1/7	FE
50/9	Barley	R	-	2.75	similar 46/5	FE
51	Barley	S	S	2.90	similar 7b/4, but paler	-
51/1	Barley	S	S	-	-	-
51/2	Barley	S	S	-	-	-
51/3	Barley	S	S	-	-	-
51/4	Barley	S	S	-	-	-
51/7	Barley	S	-	-	-	-
51/8	Barley	S	-	-	-	-
51/9	Barley	S	-	-	-	-
51/10	Barley	S	-	3.05	similar 7b/4, but greyer	FE
52/1	Barley	S	-	-	-	-
52/5	Barley	S	-	-	-	-
52/7	Barley	R	-	-	-	-
53/2	Barley	R	S	-	-	-
54/1	Wheat	S	-	3.10	similar 7b/4, but fluffier	-
54/2	Wheat	S	-	-	-	-
54/3	Wheat	S	-	-	-	-
54/5	Wheat	S	-	-	-	-
55	Wheat	S	-	-	-	-
56	Wheat	S	-	-	-	-
56/1	Wheat	S	-	-	-	-
56/2	Wheat	S	-	-	-	-
57	Wheat	S	S	-	-	-
58/5	Wheat	S	-	2.50	similar 7b/4, but whiter	FE
59/1	Wheat	R	S	2.45	large lump, similar 50/5	-
59/6	Wheat	R	S	2.60	lump similar 47/8, ring similar 50/9	FE
59/9	Wheat	S	S	-	-	-
60	Barley	R	-	2.50	large lump, white ring	FE
60/1	Barley	S	-	-	-	-
60/2	Barley	S	S	-	-	-

60/3	Barley	S	-	3.20	grey lump, many rings	FE
60/4	Barley	R	-	2.50	similar 15/2.1	FE
60/5	Barley	S	-	2.30	large lump only	-
61/1	Wheat	S	-	-	-	-
61/2	Wheat	S	-	2.60	similar 50/6	FE
61/4	Wheat	-	-	-	-	-
63/1	Wheat	S	S	-	-	-
63/2	Barley	S	S	-	-	-
63/3	Barley	S	-	-	-	-
63/6	Barley	S	-	2.65	no lump, colony high unusual rings	FE
63/7	Barley	S	-	2.30	similar 1/7	FE
63/8	Barley	S	-	2.50	similar 7b/4	FE
64/1	Barley	S	S	1.50	-	-
64/3	Barley	S	-	2.35	similar 66/3	FE
64/5	Barley	S	-	-	-	-
65/1	Wheat	S	-	3.05	similar 7b/4	FE
65/2	Wheat	S	-	3.15	wide lump, white-grey	FE
66/1	Barley	S	-	3.10	lump, colony with geen/white ring	FE
66/2	Barley	S	-	2.60	similar 66/1	FE
66/3	Barley	S	-	2.85	similar 66/1	FE
66/4	Barley	S	S	2.90	similar 66/1	FE
66/5	Barley	S	-	3.00	similar 66/1	FE
66/6	Barley	S	-	2.75	similar 66/1	FE
66/7	Barley	S	-	2.70	similar 59/6	FE

TABLE A2.2
ISOLATES - Southland survey 1984/85

ISOLATE	GROWTH TYPE	SENSITIVITY TO BENOMYL	SENSITIVITY TO PROCHLORAZ
85/1/1	FE	200B R	2P I
85/2/1	SF	0.002B R	20P I
85/2/2	SF	2B S *	2P S
85/2/3	SF	2B S *	
85/2/4	SF	2B S *	
85/2/5	SF	2B S *	
85/2/6	SF	2B S *	2P S
85/3/1	FE	0.002B R	2P S
85/3/2	-	2B R #	
85/3/3	FE	2B S *	200P I
85/4/1	FE	200B R	2P I
85/4/2	FE	200B R	
85/4/3	FE	2B R #	
85/5/1	-	2B R #	
85/6/1	FE	0.002B R	200P I
85/6/2		2B S *	2P I
85/6/3	FE	0.2B R #	20P I
85/6/4	FE	0.002B R	20P I
85/7/1	SF	0.02B R #	2P S
85/8/1	-	2B R #	
85/8/2	FE	0.02B R	2P I
85/9/1	-	2B R #	
85/9/2	-	2B R #	
85/9/3	-	2B R #	
85/10/1	FE	0.002B R	2P I
85/11/1	-	0.02B R	
85/12/1	FE	2B S *	2P S
85/13/1	-	2B S *	

not tested above this concentration
* not tested below this concentration

TABLE A2.3
ISOLATES OBTAINED FROM CEREAL SAMPLES COLLECTED FROM A FUNGICIDE FIELD TRIAL - HARVESTED BY RAHADL CONSULTANCY LIMITED FOR DU PONT (N.Z.) LTD

PLOT**	ISOLATE	GROWTH TYPE	SENSITIVITY TO BENOMYL	SENSITIVITY TO PROCHLORAZ
F	K1	FE	0.002B R	200P I
E	K2 2	FE	0.002B R	200P I
E	K2 3	FE	0.002B R	2P I
G	K4 1	-	2B S *	
B	K6 1	-	0.02B R #	
E	K8 1	FE	0.02B R	20P I
B	K13	SF	0.002B R	2P S
E	K15 1	-	0.02B R	200P I
A	K16 1	SF	0.002B R	200P I
A	K16 2	-	2B S *	20P I
A	K16 3	FE	0.02B R	20P I
A	K16 4	FE	0.02B R	2P I
A	K16 5	FE	0.02B R	200P I
A	K16 6	-	2B S *	
A	K16 7	-	2B R #	
F	K17 1	-	2B S *	
G	K18 3	FE	2B S*	
D	K19 2	-	2B S*	
C	K21 1	FE	0.02B R	200P I
E	K23 1	FE	0.02B R	200P I
G	K25 1	-	2B S *	
A	K26 1	FE	2B S	20P I
A	K26 2	-	0.002B R	
A	K26 3	-	2B S *	
C	K27 1	-	2B R #	
C	K27 2	-	0.02B R	20P I
C	K27 4	FE	0.02B R	20P I

** PLOT TREATMENT

A = untreated
B = DPXH6573 175g a.i./ha
C = DPXH6573 200g a.i./ha
D = DPXH6573 220g a.i./ha
E = DPXH6573 175g a.i./ha and Benlate 500g
F = Tilt 125g a.i./ha
G = Benlate 500g a.i./ha and Bayleton 125g a.i./ha

Plots sampled 17/1/85 - 41 days after first spray
and 13 days after second spray

not tested above this concentration
* not tested below this concentration

TABLE A2.4
ISOLATES - N.Z. SURVEY 1985/1986

ISOLATE NO.	GROWTH TYPE AND DESCRIPTION		SENSITIVITY TO BENOMYL
86/2/1	FE	-	-
86/3/1	FE	dark grey, lumpy	200B I
86/5/1	FE	grey, cream, fawn	0.2B I just, 0.02B I
86/5/2	FE	-	-
86/5/3	FE	dark grey	-
86/5/4	FE	dark grey	-
86/5/5	FE	dark grey	0.2B I pink, 0.02B I
86/5/6	SF	-	200B I
86/5/7	FE	dark grey	0.2B I pink, 0.02B I
86/5/8	FE	dark grey	0.2B I
86/5/21	FE	white with grey lump	-
86/5/28	-	-	-
86/27/1	SF	-	-
86/28/1	FE	-	-
86/32/1	FE	dark grey	0.2B I pink, 0.02B I
86/32/3	FE	dark grey	0.2B I
86/33/1	SF	grey and pink/cream	200B I 0.02B I
86/33/2	FE	dark grey	200B I 1/2 reps sporulates
86/36/1	FE	-	-
86/36/2	FE	-	-
86/36/3	FE	dark grey	0.2B I pink, 0.02BI
86/36/4	SF	grey and cream	0.02B I
86/36/5	SF	grey	-
86/36/6	SF	pale white/yellow/grey patchy pigment, see photo,	200B I conidial suspension grew on benomyl, too.
86/36/7	SF	dark grey and white	0.02B I
86/36/8	FE	-	-
86/36/9	FE	dark grey	0.02B I
86/36/10	FE	-	-
86/36/11	FE	dark grey	0.02B I
86/36/12	FE	-	-
86/40/1	FE	-	2B
86/57/A	FE	-	-
86/57/B	SF	-	-
86/57/C	FE	-	-
86/57/D	FE	-	-

86/57/5	FE	-	-
86/57/6	SF	-	-
86/57/7	SF	-	-
86/57/9	SF	-	-
86/57/11	SF	-	-
86/57/15	SF	-	-
86/57/16	SF	-	-
86/57/17	FE	-	-
86/70/1	FE	-	-
86/78/4	SF	grey and yellow	0.02B I
86/78/11	SF	grey and yellow feathers	0.02B I
86/78/13	SF	grey and white	0.02B I
86/78/18	SF	grey and white and pink/yellow	0.02B I
86/78/21	FE	brown/grey	0.02B I
86/78/22	SF	grey and pink	0.02B I
86/81/1	FE	-	-
86/81/2	FE	dark grey	0.2B I pink, 0.02B I
86/81/3	-	grey	0.2B I pink, 0.02B I
86/86/1	FE	-	2B
86/86/3	FE	-	-
86/86/4	FE	mid grey	0.2B I just
86/86/5	FE	mid grey	0.2B I pink, 0.02B I
86/86/6	FE	dark grey	0.2B I just
86/86/7	FE	dark grey	0.2B I just
86/86/9	SF	grey and white	0.02B I
86/86/10	SF	-	-
86/86/14	SF	-	-
86/86/16	FE	dark grey	-
86/86/17	FE	dark grey	0.2B I just, white, brown plug 0.02B I
86/86/18	FE	dark grey	0.2B I just
86/86/20	FE	grey	0.2B I pink, 0.02B I
86/86/22	FE	dark grey-light grey ring	0.2B I pink, 0.02B I
86/86/26	FE	dark grey	0.2B I pink, 0.02B I
86/86/27	FE	grey and white	0.2B I
86/86/30	FE	dark grey	0.2B I
86/86/31	-	-	-
86/86/33	FE	grey rings	0.2B I pink, 0.02B I
86/86/34	FE	dark grey	0.2B I pink, 0.02B I
86/86/35	SF	grey, pink/yellow/white	0.02B I
86/86/100	FE	slower, grey and green	0.02B I
86/87/1	-	-	0.02B I
86/87/2	-	-	0.02B I
86/87/3	-	-	0.2B just
86/87/4	-	-	0.2B just
86/88/3	SF	-	2BI
86/88/5	FE	-	2BI
86/88/7	SF	dark grey	2BI

86/88/8	SF	-	-
86/88/9	SF	-	-
86/88/14	SF	-	-
86/88/16	SF	-	-
86/89/5	SF	-	-
86/89/7	SF	-	-
86/89/8	SF	-	-
86/89/9	SF	-	-
86/1SC/2	FE	white/grey, yellow underneath	-
86/2SC/1	FE	-	-
86/2SC/2	FE	med. grey and cream	0.2B I pink, 0.02B I
86/2SC/3	FE	-	-
86/2SC/4	FE	yellow pigment of slow feathery	-
86/2SC/5	FE	white/grey	0.2B I
86/2SC/6	FE	-	-
86/2SC/7	FE	white/grey, yellow underneath	0.2B I
86/2SC/8	FE	dark grey	0.2B I
86/2SC/9	FE	grey and white	0.2B I pink, 0.02B I
86/2SC/10	FE	pale grey	0.2B I pink, 0.02B I
86/2SC/11	FE	white/grey, yellow underneath	0.2B I just, brown, white plug, not sporulate
86/2SC/12	FE	grey	0.2B I pink, 0.02B I
86/2SC/13	-	-	-
86/2SC/14	FE	grey/white, yellow	0.2B I pink, 0.02B I
86/2SC/15	FE	grey/white, yellow	-
86/2SC/16	FE	grey/white, yellow	-
86/2SC/30	FE	grey and white	-
86/2SC/31	FE	grey and white	0.2B I
86/2SC/35	FE	grey, white and yellow	-
86/2SC/36	-		-
86/2SC/37	FE	dark grey	0.2B just
86/NC17/1	FE	dark grey, green agar	0.2B I pink, 0.02B I

TABLE A2.5 SUMMARY OF NUMBERS OF ISOLATE TYPES COLLECTED IN ANNUAL SURVEYS

WHEAT	FE	SF	UNKNOWN	TOTAL	BARLEY	FE	SF	UNKNOWN	TOTAL	RYE	FE	SF	UNKNOWN	TOTAL	GRAND TOTAL
1983/84															
BS	17	1	26	44(84.6%)		23	1	36	60(83.3%)		0	0	2	2	106(84.1%)
BR	4	0	4	8(15.4%)		9	1	2	12(16.7%)		0	0	0	0	20(15.9%)
UNKNOWN	0	0	0	0		0	0	0	0		0	0	0	0	0
TOTAL	21(40%)	1(2%)	30(58%)	52		32(44%)	2(3%)	38(53%)	152		0(0%)	0(0%)	2(100%)	2	126

WHEAT					BARLEY										
1984/85															
BS	3	0	1	4(36.36%)		4	7	2	13(76.47%)						18(64.24%)
BR	1	0	6	7(63.63%)		3	0	0	3(17.65%)						10(35.71%)
UNKNOWN	0	0	0	0(0%)		1	0	0	1(5.88%)						1(3.57%)
TOTAL	4 (36.36%)	0(0%)	7(63.63%)	11		8(47.06%)	7(41.18%)	2(11.76%)	17						28

WHEAT					BARLEY				TRITICALE					
1985/86														
BS	33	9	4	46	2	0	1	3(12%)	0	0	0	0(0%)	49(43.36%)	
BR	2	4	0	6	3	1	0	4(17%)	2	0	0	2(100%)	12(10.62%)	
UNKNOWN	20	11	4	35	9	8	0	17(71%)	0	0	0	0(0%)	52(10.62%)	
TOTAL	55	27.59%	9.2%	87	14(58%)	9(37%)	1(4%)	24	2(100%)	0(0%)	0(0%)	2	113	

TABLE A2.6 ISOLATES FROM WHEAT COLLECTED FROM A BASF NEW ZEALAND LTD FUNGICIDE SPRAYING TRIAL IN 1985.

CHJA1/4/3	FE	grey	20B I
GHJA1/4/4	FE	grey	20B I 200B I just on plug
GHJA1/4/5	FE		
GHJA1/4/6	FE		
GHJA1/5/1	FE	dark grey, strands	200B I
GHRS1/7	FE		
GHRS1/11	FE		
GHRS1/101	SF		2B I
GHRS2/1	FE	pale, like SC2	200B I
GHRS2/8	FE	dark grey, white ring	200B I
GHRS3/B	FE		
GHRS8/A	FE	dark grey	200B I
GHJA1/4/4	FE	grey	20B I 200B I just on plug
GHJA1/4/5	FE		
GHJA1/4/6	FE		
GHJA1/5/1	FE	dark grey, strands	200B I
GHRS1/7	FE		
GHRS1/11	FE		
GHRS1/101	SF		2B I
GHRS2/1	FE	pale	200B I
GHRS2/8	FE	dark grey, white ring	200B I
GHRS3/B	FE		
GHRS8/A	FE	dark grey	200B I
GHRS8/B	FE	grey	200B I
GHRS8/C	FE		
GHRS8/D	FE	dark grey	200B I
GHRS8/E	SF	grey, fluffy	0.02B I

TABLE A2.7
OVERSEAS ISOLATES

1. U.K.

1/1	Feathery, quite fast, stromata (from 26/8)	- 2BS
1/2	Feathery, quite fast,	- 2BR
1/100	Fast Even, grey/green, sim. N.Z.	- 2BS

3. Cologne, No MBC, 10 yrs cont. wheat

3/1a	Fast Even, sim. N.Z.	- 2BS
3/1b	Fast Even, sim. N.Z.	
3/2	Slow Feathery	
3/3	Slow Feathery	- 2BR
3/4	Slow Feathery	- 2BS
3/5	Fast Even, dk grey/green	- 2BS
3/6	Fast Even, dk grey/green	- 2BS

4. U.K. triticale, R-type

4/1	Slow Feathery	- 2BR
4/2	Slow Feathery	- 2BR
4/all same lesion - 8 isolates all SF		- 2BR

5. Cologne, MBC-treated, 10 yrs cont. wheat

5/1	Slow Feathery, grey/orange, faster	- 2BR
5/2	Slow Feathery, even edge, grey	- 2BR
5/3	Slow Feathery, yellow	- 2BR
5/4	Slow Feathery	- 2BR
5/5	Slow Feathery	- 2BR
5/6	Slow Feathery	- 2BR
5/7		

APPENDIX 3 Electrophoresis

TABLE A3.1

STOCK SOLUTIONS FOR ELECTROPHORESIS

A.	1N HCl	48ml
	TRIS	36.6g
	TEMED	0.23ml
+	Water to	100ml
	pH = 8.9	
B.	1N HCl	approx. 48ml - pH adjusted by titrating with 1N HCl
	TRIS	5.98g
	TEMED	0.46ml
+	Water to	100ml
	pH = 6.7	
C.	Acrylamide	28.0g
	BIS	0.735g
+	Water to	100ml
D.	Acrylamide	10.0g
	BIS	2.5g
+	Water to	100ml
E.	Riboflavin	4mg
+	Water to	100ml
F.	Sucrose	40g
+	Water to	100ml

Working solutions

Four small-pore main gels (8%)

1ml A + 2ml C pH 8.9 (8.8-9.0)

The solution is degassed and added to 32ml ammonium persulphate (0.014g/l)

Large-pore stacking gel

0.5ml B + 2ml D + 1ml E + 4ml F

pH 6.7 (6.6-6.8)

APPENDIX 4 - PATHOGENICITY TRIALS - INFECTION SCORES

TABLE A4.1

LINCOLN CULTIVAR FIELD TRIAL

REPLICATE 1

BLOCK 1 - 2C

6	8	6	0	0	3	2	0	7	0	0	0	6	1	0	6	4	2	2	0
6	6	3	0	7	7	7	0	4	1	3	2	6	3	6	5	0	2	4	2
6	7	6	7	7	4	5	2	0	0	6	7	7	5	7	7	7	6	7	7
8	4	0	0	1	0	1	7	0	0	1	0	0	5	2	6	0	7	1	8
7	0	0	6	1	5	0	4	0	1	0	7	0	4	7	0	3	2	7	6
2	2	0	0	4	2	3	3	2	3	7	7	3	7	1	4	3	3	6	2
6	3	2	7	6	0	8	3	4	6	5	5	8	0	0	0	4	5	4	0
8	7	6	1	5	7	4	0	1	0	5	7	4	7	0	6	7	0	0	6
5	5	4	0	0	6	6	3	0	0	0	5	0	0	4	6	2	3	4	7
2	3	7	4	4	5	0	6	4	2	2	0	0	1	0	2	0	7	2	3
0	1	0	0	0	2	2	7	2	0	0	2	1	7	1	7	7	7	3	2
7	7	1	7	6	6	7	2	3	0	2	2	2	2	5	1	1	3	7	2
4	6	4	2	6	7	2	2	0	7	8	4	4	8	6	7	8	7	2	3
4	4	0	5	7	5	2	0	0	2	5	0	0	0	0	0	0	0	0	0
0	0	5	7	6	4	1	5	6	6	6	0	0	0	5	6	7	0	7	7
0	0	0	3	7	0	0	2	6	7	5	7	5	8	7	6	7	7	2	0
6	0	2	0	4	0	3	0	0	3	0	6	0	0	0	0	7	7	2	0
0	0	0	0	4	6	0	0	3	0	0	0	0	0	0	0	3	2	0	0

BLOCK 2 - 3A

7	7	6	7	2	7	4	2	2	7	5	7	7	6	4	4	8	8	7	7
3	6	7	4	4	2	2	3	7	3	6	3	7	7	6	6	8	4	6	4
7	7	3	3	6	6	6	6	7	7	7	8	4	4	4	4	6	7	6	6
3	7	0	7	7	4	4	7	4	6	2	2	3	4	6	7	6	7	6	3
6	6	0	4	2	7	0	6	6	6	7	5	6	4	6	0	6	3	3	4
7	7	7	7	7	6	4	7	7	7	7	8	4	6	7	7	6	8	4	7
6	4	7	7	6	7	4	4	7	5	5	7	4	7	7	7	7	7	2	8
7	6	7	7	7	7	7	6	7	7	7	7	6	7	7	7	7	6	7	8
3	7	6	7	7	2	1	3	6	7	4	4	7	7	7	7	4	6	6	6
2	0	4	2	0	4	2	7	4	0	2	0	0	2	0	5	0	3	5	0
5	6	0	6	0	8	7	0	2	2	0	0	0	0	8	3	0	7	7	0
6	5	5	6	6	3	7	7	7	2	7	6	8	7	5	6	7	6	8	7
7	7	6	6	3	6	1	2	7	6	7	7	7	7	7	6	7	6	7	7
7	6	7	5	8	8	6	3	7	6	7	*	*	*	*	*	*	*	*	*
2	6	7	0	6	7	6	6	6	7	6	7	6	4	4	7	7	7	6	6
6	7	8	7	6	4	7	7	6	7	7	6	6	7	7	7	6	6	6	8
7	7	7	2	4	5	5	4	0	0	0	4	6	0	7	4	7	1	7	0
6	3	6	4	7	7	7	6	5	7	6	7	4	7	7	7	4	7	7	7

BLOCK 3 - 1B

6 7 7 8 7 7 5 7 6 6 2 2 7 6 7 7 6 2 4 7
8 6 7 7 7 7 7 6 7 6 6 6 7 6 6 7 7 6 7 6
7 5 7 3 5 7 4 7 6 7 7 7 6 3 7 7 8 8 8 7
7 6 7 7 7 7 6 7 8 7 7 7 7 7 7 6 7 7 7 7
7 6 3 3 3 4 7 7 6 6 7 0 8 6 2 3 7 5 3 8
6 5 7 7 6 6 6 3 6 6 6 7 6 4 6 6 3 6 5 6
8 5 6 6 3 7 2 6 8 7 8 7 4 7 6 8 6 8 6 6
6 6 6 2 7 5 6 8 6 3 6 8 5 5 8 6 6 7 5 8
7 7 4 7 7 6 6 3 6 6 6 6 7 5 7 7 4 5 3 3
4 6 7 7 6 7 6 7 6 7 8 7 7 7 7 7 6 6 6 7
7 7 5 3 7 6 6 7 6 7 7 7 6 6 4 7 5 6 3 2
4 4 7 4 4 4 7 3 7 7 4 4 7 3 7 4 7 4 7 6
2 2 7 5 7 5 6 3 7 4 7 7 3 2 7 6 7 5 3 2
7 5 4 8 7 5 6 6 5 7 4 4 4 6 8 3 4 8 7 6
7 7 6 8 7 8 7 7 7 8 8 7 7 7 7 7 4 7 8 7
2 3 4 7 7 4 3 0 3 4 5 4 3 2 3 4 7 3 8 4
7 6 7 4 8 8 7 2 7 7 7 7 7 4 7 2 4 8 7 7
5 7 0 6 6 6 7 7 3 7 6 7 3 6 7 8 4 8 8 6

REPLICATE 2

BLOCK 1 - 4B

7 5 8 6 7 7 5 7 6 7 7 7 7 7 6 7 7 8 7 *
7 6 7 7 7 6 6 7 7 7 7 6 7 8 7 6 8 6 2 *
7 6 6 6 2 5 4 6 7 7 7 7 7 7 6 7 2 7 7 7
6 7 7 2 6 7 7 7 6 7 7 7 7 7 6 7 7 6 7 7
7 7 7 7 6 7 6 6 7 6 6 7 6 3 5 6 7 7 7 6
7 6 6 7 7 6 7 7 7 8 6 7 7 7 7 7 7 7 7 7
6 7 7 7 6 5 6 7 6 6 6 6 6 7 5 6 6 6 7 6
7 6 7 7 7 7 7 8 7 7 7 7 7 7 8 7 6 8 7 7
7 7 6 7 7 7 7 7 4 7 6 6 7 7 6 6 6 7 6 *
7 5 4 8 4 5 7 7 4 6 7 6 4 6 6 7 5 6 8 8
4 7 4 6 3 8 2 0 8 5 8 4 3 5 4 4 4 5 4 4
7 6 7 7 7 7 7 8 7 2 8 7 7 7 4 6 5 2 7 7
5 5 4 6 8 4 4 6 8 7 4 3 1 8 5 3 5 4 4 4
6 4 8 8 7 7 7 5 6 7 8 7 7 5 8 8 6 5 8 6
8 7 6 5 7 7 6 5 7 8 7 8 4 4 6 8 5 7 6 6
8 7 3 7 8 8 8 8 8 8 4 5 8 1 8 8 3 2 6 7
4 8 5 8 7 5 4 6 2 2 7 4 3 5 7 1 3 4 7 8
4 6 7 7 6 4 7 7 7 5 7 7 4 7 7 7 7 7 7 5

BLOCK 2 - 1A

7 7 7 7 6 5 6 7 7 7 5 2 7 5 7 4 7 8 6 7
7 7 7 6 7 3 7 7 6 6 7 7 7 6 7 7 6 4 7 8
7 6 6 7 7 7 6 7 7 7 7 6 7 7 8 7 7 6 7 7
7 6 7 7 5 6 5 7 6 6 7 6 6 6 6 7 7 7 8 7
6 7 8 7 7 7 7 7 7 7 6 7 7 7 7 7 6 7 6 6
6 6 7 8 6 7 7 4 2 7 6 5 6 6 6 5 7 7 6 6
6 7 7 7 7 7 7 7 8 7 7 8 6 8 6 7 7 8 7 8
8 8 7 8 * * * * * * * * * * * * * * * *
6 7 5 4 8 5 0 5 4 4 8 6 6 7 7 5 6 7 5 7
5 7 8 4 7 7 6 6 6 6 4 7 7 5 8 8 8 7 8 7
6 6 6 6 6 7 7 2 6 6 7 6 7 5 7 3 6 5 6 6
3 8 4 4 3 6 7 5 7 6 8 8 5 7 7 7 7 4 6 7
8 7 8 7 7 7 7 5 4 7 6 5 8 7 7 8 7 8 3 7
7 3 3 7 6 7 5 7 6 5 3 5 4 7 0 4 4 7 2 8
7 8 8 6 7 7 6 7 6 6 4 7 7 6 7 7 2 7 7 7
8 7 8 7 7 7 8 7 5 7 7 4 4 4 7 7 6 7 7 5
2 7 7 3 3 2 8 6 8 7 7 6 7 4 4 4 4 6 6 6
4 6 4 5 7 6 6 6 5 4 7 4 7 7 6 7 3 4 7 4

BLOCK 3 - 3C

5 3 6 3 4 4 3 7 2 5 6 5 7 7 6 4 6 3 3 7
0 3 7 7 7 0 2 7 0 6 6 7 7 6 2 6 7 5 6 0
6 5 6 5 5 7 6 7 6 7 7 7 7 7 7 7 5 5 7 6
8 7 7 7 2 7 7 7 7 7 7 7 7 2 4 8 5 7 6 7
7 5 0 6 6 8 8 7 7 8 8 3 8 8 8 8 7 8 8 8
3 1 6 6 3 6 4 2 7 6 2 6 3 6 2 1 7 2 2 2
4 6 1 6 7 1 3 3 7 5 2 6 3 6 7 7 1 6 0 1
6 4 3 2 2 3 2 7 7 2 8 6 6 2 3 3 2 2 7 3
2 0 0 2 2 0 7 0 0 7 3 5 0 3 6 5 4 0 7 5
2 7 7 6 6 7 5 7 6 7 6 5 7 7 6 7 8 4 8 6
6 5 7 5 7 2 3 5 7 6 6 7 7 7 7 5 7 6 7 2
7 4 2 7 4 3 7 4 6 8 7 2 4 4 5 3 5 4 3 2
5 1 2 7 6 7 2 7 7 6 4 5 7 7 4 0 2 6 5 6
3 3 3 2 2 4 3 0 4 5 3 4 8 3 4 8 3 4 4 4
3 4 2 5 0 4 5 2 0 5 6 2 3 0 5 6 3 2 7 3
3 1 7 6 6 2 4 4 7 2 3 2 7 3 0 6 6 7 7 2
1 7 2 3 2 0 2 0 0 7 2 2 3 6 2 2 3 3 2 6
1 4 5 2 7 0 4 4 2 5 4 8 2 4 2 7 4 0 7 3

REPLICATE 3

BLOCK 1 - 1C

6 7 7 7 6 6 6 7 7 7 7 7 6 7 6 6 3 8 6 3
7 0 7 0 3 4 0 0 5 2 2 0 6 2 5 3 2 5 5 0
3 6 7 7 7 7 7 6 5 6 7 7 7 7 6 5 5 7 5 7
7 7 7 3 7 7 7 7 5 8 5 7 7 6 5 0 7 7 7 7
0 2 6 5 6 6 6 5 6 6 3 7 7 3 3 7 6 7 7 3
2 3 6 3 5 4 7 7 3 4 7 3 7 3 4 3 0 2 6 7
7 6 1 4 7 4 7 3 1 0 0 0 7 2 0 7 2 7 0 5
7 3 2 3 3 4 7 7 5 5 0 6 7 7 6 2 7 3 7 1
5 6 6 6 7 6 7 7 6 6 3 7 7 2 5 7 6 7 7 7
5 6 2 3 0 4 6 7 8 5 7 7 5 0 7 3 6 3 8 6
3 5 6 3 6 3 3 6 8 6 6 8 2 8 8 8 8 8 2 8
7 8 0 7 7 7 5 4 3 6 3 4 6 5 4 5 4 4 4 7
1 4 2 0 3 3 2 2 8 6 0 3 3 0 3 3 0 0 0 0
4 4 7 7 7 4 5 3 4 4 4 6 7 5 4 4 7 8 6 6
2 0 7 3 0 2 8 7 4 2 6 4 7 4 2 3 4 5 7 2
5 7 5 7 5 6 4 2 6 0 7 4 6 4 3 4 6 4 5 3
6 6 7 6 2 6 5 6 2 2 6 1 6 3 6 7 5 5 6 2
7 8 3 2 7 6 7 5 2 4 3 2 4 6 2 2 2 3 0 6

BLOCK 2 -4B

5 6 7 7 4 6 6 3 5 6 7 6 7 7 7 5 7 7 4 8
7 6 7 7 7 7 7 7 7 8 8 7 7 7 7 7 7 8 7 7
7 7 7 7 7 7 6 7 6 7 8 8 7 7 6 7 6 6 7 6
7 7 7 7 3 4 6 8 5 6 4 8 3 4 4 3 4 3 7 8
7 7 7 7 7 7 7 8 4 6 6 7 6 7 7 8 6 7 7 7
7 7 6 8 7 7 6 7 7 7 6 6 7 6 4 7 6 6 7 7
4 7 7 5 6 7 7 7 7 7 6 6 7 7 2 5 7 7 6 7
8 7 7 7 7 8 7 3 8 8 8 7 7 8 6 7 8 7 7 8
6 7 4 5 6 7 4 5 4 7 6 5 5 0 6 7 7 7 7 5
8 8 6 7 7 6 6 7 7 7 7 3 7 7 4 7 6 7 7 7
8 7 7 7 8 4 8 7 8 8 7 7 8 8 7 7 5 7 6 7
7 7 7 7 5 7 5 6 6 7 7 7 7 5 2 7 7 7 6 7
6 4 7 7 6 7 5 7 4 7 4 7 6 7 4 8 7 3 5 8
7 5 4 7 3 7 8 4 4 4 3 4 8 6 6 4 3 3 4 6
4 7 7 7 7 7 7 7 7 8 4 7 3 5 7 7 7 4 3 7
6 7 6 5 8 8 7 6 7 7 6 6 6 7 6 6 7 7 7 7
8 7 8 6 7 7 4 7 8 7 7 7 7 8 7 7 7 7 8 7
7 6 0 7 7 7 4 4 6 7 6 7 4 7 7 7 7 4 4 7

BLOCK 3 - 2A

4 0 6 7 5 7 5 7 7 0 7 1 6 7 7 6 7 6 7 6
7 7 7 8 5 6 4 7 5 8 8 8 7 7 7 7 7 6 8 7
7 7 7 7 7 7 6 7 6 7 5 7 5 7 8 5 5 7 7 7
7 7 8 7 6 7 6 7 7 6 6 7 7 7 6 6 7 6 7 7
7 7 7 7 6 8 6 6 6 7 7 7 7 8 5 7 8 7 8 7
8 8 7 6 7 7 7 7 7 7 6 7 6 7 7 7 8 6 7 7
7 7 8 8 7 7 7 7 7 8 7 8 7 7 8 8 7 7 7 7
7 8 7 7 8 7 8 7 * * * * * * * * * * * *
7 6 7 6 7 2 6 6 7 6 6 5 5 7 7 7 5 6 6 7
6 5 7 7 7 7 7 7 7 6 7 6 6 7 7 6 7 5 6 7
7 8 2 2 0 6 0 8 3 8 8 3 5 8 5 6 5 6 7 6
7 6 7 3 6 3 8 7 8 4 7 8 6 7 3 7 7 6 7 6
5 8 7 6 4 6 7 7 7 4 7 7 6 7 4 3 7 7 7 5
3 3 8 3 3 7 5 3 3 3 7 8 7 3 4 2 0 8 7 2
7 5 8 8 5 7 6 7 5 6 7 6 7 7 7 7 7 7 7 5
7 7 2 6 6 8 6 4 7 5 7 7 7 7 7 7 5 7 7 7
2 7 7 7 7 6 7 6 8 7 7 7 6 3 7 7 6 7 3 6
7 7 7 7 7 7 7 7 7 6 7 3 7 7 7 8 7 7 8 7

REPLICATE 4

BLOCK 1 - 3A

7 6 7 5 8 7 7 7 7 8 7 7 7 7 7 7 8 7 7 7
6 7 7 7 7 7 8 7 7 7 7 7 7 7 6 7 6 8 7 7 7
7 6 6 7 7 6 7 6 7 7 7 7 7 7 7 5 7 7 6 7 7
7 7 7 7 8 8 7 7 7 7 8 6 5 6 6 7 6 4 8 7
6 6 6 8 7 7 6 7 6 6 6 6 5 7 5 6 2 7 8 0
7 6 7 7 7 6 2 6 7 6 8 6 6 7 7 7 7 7 6 7
6 7 6 7 7 8 6 6 7 7 7 7 6 7 6 7 7 6 7 6
6 7 7 7 7 7 7 8 6 7 8 8 7 6 7 7 8 7 7 7
6 5 3 7 7 6 6 7 7 6 6 7 7 7 7 5 7 5 6 6
7 7 8 7 7 6 7 7 8 2 6 7 7 7 7 8 7 7 7 8
5 7 4 7 6 8 3 6 4 7 7 8 6 8 6 7 7 5 7 7
8 7 7 8 6 3 7 7 7 7 7 8 8 8 6 7 2 7 4 3
8 6 8 8 8 6 6 7 0 7 7 7 8 8 8 6 6 6 8 7
5 5 5 7 3 8 8 8 8 6 7 7 3 6 2 8 6 4 5 8
8 5 7 3 7 7 7 5 4 8 6 7 7 4 6 6 6 6 7 7
6 5 3 7 4 6 8 0 6 3 7 7 7 6 6 7 7 6 6 6
5 7 8 6 6 6 7 7 6 5 3 8 7 7 6 8 7 8 4 6
6 6 7 7 7 7 4 5 4 4 8 7 7 4 7 8 7 7 7 6

BLOCK 2 - 2B

7 3 7 6 7 6 7 7 7 6 7 6 7 6 7 8 6 6 8 7
7 7 8 7 6 7 7 7 4 7 7 7 7 7 7 6 7 6 6 7
6 6 6 7 5 7 7 6 7 7 7 7 5 8 8 8 6 6 7 7
8 7 7 7 7 7 6 7 7 7 7 7 7 7 7 7 7 6 7 7
7 4 4 6 8 8 8 8 6 7 7 4 8 7 5 7 8 7 8 8
4 6 6 6 6 7 7 6 7 7 7 6 6 7 3 7 7 6 6 6
7 6 7 7 1 6 7 7 8 7 4 7 7 7 7 8 7 7 7 7
7 3 4 2 5 3 6 3 7 3 7 0 7 2 7 6 6 7 3 0
4 7 6 3 5 7 7 6 7 7 6 7 6 3 7 6 6 7 6 6
7 7 7 7 7 8 6 7 7 8 7 8 7 7 8 8 6 8 6 7
2 7 8 8 7 8 8 8 7 7 6 8 8 7 6 6 8 8 8 7 8
7 7 7 6 6 6 7 7 7 7 6 7 7 7 7 7 7 7 7 8
3 3 6 7 6 7 7 6 5 7 6 7 7 7 7 7 6 6 3 7
7 6 6 5 6 6 7 5 0 7 7 7 6 7 7 7 6 4 7 7
7 7 7 7 7 7 7 7 8 7 7 3 7 7 6 7 6 4 8 7
4 2 4 4 2 4 2 2 2 3 3 7 7 3 2 2 2 2 0 6
7 7 6 7 8 4 8 4 5 8 6 7 6 7 8 6 8 6 7 5
8 7 7 7 4 3 7 7 6 4 7 7 7 6 7 7 7 8 6 7

BLOCK 3 - 4C

7 3 7 2 6 7 7 4 6 7 6 5 6 7 4 7 6 6 7 6
6 3 3 3 3 7 7 2 3 6 3 0 3 5 0 6 3 0 3 3
5 0 2 7 5 4 2 3 0 2 2 0 2 2 3 3 2 3 7 3
2 7 2 0 2 3 0 1 2 0 2 0 2 2 2 2 0 2 2 3
0 2 6 0 2 5 2 5 1 7 2 3 7 4 2 2 2 0 7 3
5 7 5 5 6 6 6 7 7 6 7 6 6 4 5 7 7 5 6 5
6 4 7 6 5 7 5 7 5 7 7 7 7 7 7 6 6 7 6 7
5 6 7 5 6 0 2 7 7 6 0 5 6 6 6 0 0 0 7 7
0 7 6 6 7 5 0 0 3 2 3 7 1 7 6 3 0 0 6 2
6 0 7 6 5 6 2 7 7 7 7 7 6 4 8 2 0 7 6 2
0 0 0 0 2 3 3 0 0 0 2 2 2 0 3 0 2 2 4 0
8 7 7 5 2 7 3 4 7 0 4 2 8 0 8 2 7 2 3 2
7 6 4 4 5 7 7 0 7 6 6 0 6 7 7 0 6 5 6 7
5 2 7 7 6 6 7 7 2 2 3 6 7 7 2 6 6 0 2 2
5 0 6 7 7 7 6 6 7 6 6 2 7 5 7 7 2 7 6 7
3 8 6 6 0 2 7 8 6 0 5 6 0 2 6 6 5 4 5 2
4 3 3 2 2 0 3 3 7 8 6 5 6 6 3 3 0 2 7 0
4 4 3 3 3 3 2 3 6 6 3 2 2 3 2 4 8 2 2 2

REPLICATE 5

BLOCK 1 - C1

3 4 2 3 3 0 0 3 7 0 0 3 0 2 5 0 0 7 2 2
1 2 1 0 0 6 0 1 3 0 3 1 0 0 0 2 4 1 0 0
0 4 0 0 7 2 2 0 7 0 0 3 0 1 0 0 0 0 5 1
0 2 3 4 0 4 2 6 2 0 7 0 4 7 2 0 3 6 0 3
0 0 0 0 0 1 2 3 0 0 0 0 0 1 0 0 2 2 3 6
2 3 0 2 0 0 0 0 0 4 0 2 1 0 2 0 0 7 7 0
6 0 3 6 6 5 5 0 7 8 6 8 0 4 7 8 0 2 6 2
2 0 3 4 3 1 0 2 0 0 0 0 3 2 7 2 2 2 2 0
0 6 0 0 2 4 2 2 0 7 7 0 6 3 7 0 5 0 7 7
7 4 6 6 6 3 0 0 0 0 0 0 0 7 6 7 0 0 0 0
0 1 3 0 0 0 6 3 0 0 3 2 0 0 4 3 0 4 2 8
0 0 0 4 0 8 3 2 0 3 0 6 4 2 3 6 5 0 3 0
6 3 0 0 6 6 5 4 7 0 5 4 7 0 5 0 2 0 5 1
0 0 6 3 5 0 4 3 0 0 0 2 2 5 2 2 0 4 0 2
3 3 2 0 2 2 0 2 3 6 2 3 3 2 7 3 5 5 7 6
0 5 7 6 2 0 4 2 3 2 6 3 0 6 3 3 3 0 6 8
0 0 5 0 0 0 0 2 0 6 5 0 3 2 2 2 0 0 2 2
0 0 0 0 2 2 4 0 0 0 0 0 6 0 0 0 0 0 0 0

BLOCK 2 - B3

6 7 7 6 6 6 8 6 7 7 7 7 8 6 6 7 6 6 7 7
6 6 7 6 6 6 5 7 6 3 3 7 6 6 4 7 5 6 7 6
6 6 6 7 6 7 6 6 7 6 6 2 6 7 6 7 6 4 6 7
7 7 7 6 7 7 6 7 7 7 7 7 7 7 7 7 7 7 6 8
5 7 7 6 7 7 7 8 7 7 7 8 6 7 7 7 8 8 8 7
6 7 7 6 7 5 7 7 0 7 7 7 7 7 6 7 7 6 7 7
7 7 7 7 7 7 7 7 7 7 7 7 7 6 8 7 7 7 7 7
7 7 7 5 7 6 7 7 7 6 7 6 7 8 7 7 6 8 6 7
7 7 7 7 7 8 7 7 7 7 7 6 8 7 8 7 7 7 7 8
7 7 7 7 7 7 7 6 7 7 7 7 7 7 7 8 7 7 7 7
7 7 7 6 6 6 6 7 5 7 6 8 7 3 7 7 7 7 6 8
5 7 7 6 8 7 8 6 8 7 6 7 7 5 6 7 6 8 7 7
7 6 4 8 4 6 6 8 6 5 6 6 4 6 6 6 5 3 5 6
7 7 7 4 6 3 6 7 7 6 6 7 6 6 6 7 7 7 7 7
4 6 6 6 6 8 6 3 6 3 6 6 8 6 4 6 4 6 6 6
7 7 7 3 6 0 7 5 7 7 7 3 3 7 2 7 8 3 0 5
6 6 6 2 5 6 7 7 7 7 5 7 5 4 7 4 7 6 7 7
4 0 4 3 4 7 6 3 5 5 4 3 4 0 6 7 7 6 3 1

BLOCK 3 - A2

6 7 7 6 7 7 4 6 7 0 7 1 6 7 7 2 6 5 7 1
6 7 7 2 6 7 6 7 6 7 8 7 7 7 7 7 7 7 7 7
8 6 7 7 6 6 8 8 6 7 7 7 6 7 7 7 7 8 6 7
7 7 6 7 8 7 7 7 7 7 6 7 3 7 7 7 7 7 7 7
6 6 7 2 7 7 6 2 6 7 7 6 7 1 7 6 6 6 7 7
7 7 7 8 7 7 7 7 8 7 7 7 7 7 7 7 7 7 8 6
6 7 6 7 6 6 7 6 7 7 6 6 6 6 6 7 6 6 7 6
7 7 8 7 7 7 7 7 3 8 4 8 7 8 8 8 8 7 7 7
6 7 6 7 4 6 7 6 6 7 7 7 7 7 7 7 6 7 7 6
6 7 7 7 7 5 4 4 7 6 4 2 3 7 3 4 3 7 4 6
6 6 6 5 6 6 7 6 7 7 7 6 3 3 5 5 4 6 5 4
7 7 7 5 5 7 7 5 5 5 3 8 6 5 2 5 5 8 8 7
6 4 5 6 4 2 6 6 7 6 3 5 6 7 3 6 6 4 5 2
7 5 6 6 4 4 4 7 6 4 4 6 7 7 4 6 6 6 4 6
8 7 7 7 7 7 7 6 7 7 7 7 7 7 7 7 7 7 7 7
7 6 7 7 6 7 7 8 0 7 8 6 7 7 7 7 6 6 7 6
4 2 5 6 5 6 7 6 3 7 7 8 6 7 7 7 7 7 5 4
4 6 7 8 3 8 8 7 8 4 4 7 6 7 6 7 7 8 5 5

TABLE A4.2 - GORE CULTIVAR FIELD TRIAL

INCOMPLETE LATIN SQUARE DESIGN

BLOCK 1 - 1A

0	3	4	3	6	6	0	2	0	7	2	7	7	0	3	2	0	7	3	6
7	8	7	6	7	6	6	7	7	7	7	7	6	7	7	7	6	6	7	8
6	7	5	7	2	6	8	7	7	4	0	6	6	7	7	6	2	7	7	7
6	6	6	6	0	0	6	2	2	7	7	2	2	6	6	2	0	2	2	6
7	7	0	4	0	7	7	7	0	0	7	7	7	6	1	0	0	7	7	5
0	6	2	3	7	0	4	6	0	6	0	0	5	6	7	6	7	6	6	6
0	0	7	0	3	0	2	6	6	0	0	3	7	0	7	5	6	0	0	0
6	6	7	2	2	0	7	7	7	6	7	6	8	7	2	7	7	7	3	7
5	0	7	2	3	6	5	7	0	5	2	0	0	0	0	3	6	0	6	0
6	7	7	7	7	3	4	7	6	7	7	0	2	4	7	6	7	7	5	6
5	7	7	7	7	6	7	6	2	7	2	7	6	7	5	5	6	7	5	2
7	0	4	2	1	0	2	6	5	0	7	6	7	2	0	0	0	7	7	7
0	7	4	3	7	6	6	8	7	6	0	6	7	7	4	6	0	2	3	3
0	0	0	0	0	5	2	2	7	0	0	0	0	4	6	0	0	0	0	0
5	0	0	0	7	0	0	0	7	0	0	0	6	5	2	0	0	4	4	4
3	2	4	6	3	2	2	2	0	4	2	7	0	0	2	2	2	1	0	2
6	7	7	6	3	7	3	6	6	7	7	6	7	7	7	7	7	7	7	7
5	6	5	6	7	5	7	6	6	7	5	5	7	5	6	7	7	0	4	6

BLOCK 2 - 3B

7	7	3	7	2	6	6	7	6	2	6	7	7	7	6	6	6	6	7	2
7	7	6	6	7	6	5	7	7	7	6	7	7	6	6	7	6	6	6	3
5	7	5	6	7	7	7	7	7	0	7	7	7	7	7	5	5	7	7	7
7	8	7	6	7	5	6	0	2	7	6	7	5	7	6	7	6	2	4	6
6	5	7	6	0	6	3	6	7	6	7	3	7	2	3	5	6	0	6	2
5	0	5	3	3	3	5	2	3	7	7	5	3	3	5	4	0	4	7	3
2	5	6	4	3	4	7	7	7	7	3	4	7	5	7	3	5	7	7	7
6	2	0	5	6	1	6	0	1	0	7	2	3	2	0	0	0	0	0	0
5	6	5	0	4	7	0	0	6	6	5	3	6	6	6	6	6	7	6	8
5	5	5	7	0	5	4	7	6	0	5	5	0	7	5	3	4	8	6	5
6	7	6	7	3	8	2	5	7	5	3	3	6	6	7	2	7	0	4	0
4	5	6	3	7	3	7	7	6	3	0	2	5	0	6	5	5	7	6	2
0	2	7	0	7	1	0	1	0	0	2	3	2	7	7	0	6	1	0	0
0	0	6	7	2	4	2	2	7	2	6	4	6	2	6	0	2	6	7	7
0	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0
0	0	0	5	5	5	6	6	6	6	6	3	5	2	3	0	6	5	7	2
7	7	7	5	7	7	6	3	0	5	6	7	6	0	7	7	7	7	6	7
7	7	6	2	0	7	3	0	4	7	6	3	7	7	4	3	7	3	5	1

BLOCK 3 - 2C

0 0 3 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0
3 0 3 0 2 2 0 0 0 0 3 0 2 0 0 0 0 2 2 0
0 0 0 0 0 0 3 0 0 2 0 0 0 0 0 0 0 3 0 0
0 0 0 0 0 7 3 5 7 2 0 0 0 0 2 0 2 0 2 3
5 0 0 4 0 0 0 0 0 0 3 8 0 3 0 0 0 2 0 0
0 0 2 0 0 6 3 0 0 0 0 0 0 0 0 3 0 0 0 4
0 0 0 0 2 0 0 0 0 0 0 3 0 6 2 0 0 0 0 0
6 2 2 0 0 6 5 2 0 0 0 0 0 4 0 0 0 0 3 3
0 7 0 0 0 7 0 3 0 0 2 0 1 0 0 0 0 0 0 0
0 0 2 3 0 0 0 0 0 2 3 2 0 0 0 0 3 0 0 0
0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 2 2 0
2 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0
3 3 2 4 7 2 2 2 1 2 * * * * * * * * * *
* * * * * * * * * * * * * * * * * * * *
2 3 4 2 4 3 0 2 3 0 3 6 3 3 0 3 3 3 2 3
0 0 6 0 0 0 2 0 2 7 0 2 0 0 0 0 0 3 0 2
0 0 5 0 0 6 3 0 0 0 0 4 0 0 0 0 0 0 5 0
0 0 0 0 0 0 0 0 0 0 0 6 0 0 2 2 2 0 0 0

BLOCK 4 - 4A

0 0 2 2 3 0 0 0 2 0 0 2 0 3 2 2 0 0 0 0
0 0 0 2 0 3 2 2 0 3 0 0 0 2 0 0 0 0 2 2
0 0 0 0 0 0 0 2 4 6 0 0 0 2 0 0 0 0 0 0 0
6 3 7 4 0 7 6 5 2 0 2 2 2 0 6 7 4 0 0 0
0 0 0 0 0 0 0 7 3 2 7 0 0 0 0 0 0 0 2 2
2 0 2 7 6 7 0 3 2 0 7 5 3 3 0 4 0 2 0 0
0 0 2 2 0 0 3 0 2 3 0 0 0 0 3 0 0 0 2 0
2 0 0 0 0 8 3 0 2 3 0 0 4 6 0 0 0 0 0 0
2 0 0 3 2 3 0 0 0 0 0 0 3 3 1 0 0 0 0 0
0 0 0 2 2 5 0 2 0 6 0 0 7 4 0 0 0 4 0 2
0 7 0 0 2 0 3 0 0 3 0 3 0 7 0 0 0 2 8 0
0 2 3 0 0 0 2 0 2 3 3 0 6 0 0 2 0 4 0 0
0 0 7 7 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0
0 6 2 0 0 7 0 0 0 2 0 0 2 3 2 6 0 0 0 0
2 0 0 0 0 6 0 0 0 0 0 0 0 1 3 0 1 0 0 0
0 2 0 0 3 0 0 0 0 0 0 0 0 0 2 0 2 0 0 0
0 2 0 0 5 0 0 6 0 0 0 2 7 6 0 4 0 0 0 3
2 8 3 2 0 1 2 6 2 0 2 2 0 0 0 0 3 2 2 7

BLOCK 5 -3C

3 0 3 0 5 6 7 6 2 3 4 6 6 5 0 2 2 3 3 4
7 7 7 7 7 7 5 7 7 5 6 7 7 6 7 7 7 7 7 7
7 7 7 7 7 6 7 7 7 6 7 6 7 7 7 8 7 7 3 7
7 7 6 7 7 6 4 7 5 0 6 5 0 7 2 0 6 6 5 3
7 7 0 7 0 7 7 7 7 7 7 7 7 7 7 2 7 7 7 7
0 0 7 7 7 0 6 0 0 7 0 5 7 0 7 7 0 0 0 7
3 7 7 0 4 3 5 0 7 6 7 3 6 7 7 6 6 5 0 6
7 7 7 7 7 7 7 7 8 7 6 7 7 7 7 7 7 7 7 7
5 6 6 4 6 3 5 3 4 4 7 7 2 3 5 5 7 3 4 0
7 2 7 0 7 7 8 7 7 7 7 7 6 5 7 7 7 5 7 7
7 7 4 7 7 2 5 6 7 7 7 7 7 6 3 4 7 7 6 7
7 0 7 7 6 0 6 7 7 7 7 7 7 0 6 6 0 7 7 7
6 7 6 7 7 6 8 5 6 6 7 3 6 6 7 7 7 7 5 7
0 4 0 0 6 0 0 0 0 7 1 0 6 0 1 0 0 0 0 0
0 0 0 0 6 7 5 6 7 7 6 6 7 6 4 7 0 0 6 7
2 0 6 0 0 0 6 2 6 0 7 5 1 0 0 7 7 0 0 6
7 7 7 7 6 7 7 7 7 7 7 7 7 6 7 7 7 7 7 7
4 6 4 7 7 4 7 6 7 3 3 7 0 4 7 7 7 7 6 6

BLOCK 6 - 1A

0 3 0 0 0 7 7 6 0 7 7 7 7 7 7 7 5 0 7 7
7 7 7 7 7 7 7 7 8 7 7 7 7 7 3 6 6 7 6 7
6 5 7 7 6 7 7 7 6 6 7 6 7 7 6 6 6 7 5 7
7 7 7 6 6 6 6 7 6 7 4 6 5 0 7 7 7 8 7 7
7 6 7 7 4 6 5 7 7 7 7 6 7 3 5 5 5 3 7 6
7 7 7 0 6 7 2 5 7 7 7 7 7 7 7 7 7 7 7 7
5 5 5 0 3 5 7 7 5 3 6 4 5 5 3 6 3 2 0 3
3 4 6 4 6 6 6 7 6 2 7 7 7 6 6 6 3 5 6 6
0 6 4 4 6 3 5 6 5 6 5 5 5 0 4 7 7 7 5 3
5 4 5 0 6 7 0 6 0 0 6 6 0 6 7 3 7 3 5 0
0 0 8 7 7 7 7 1 7 6 7 0 7 0 1 7 7 7 7 7
7 7 7 7 7 6 7 6 7 7 7 7 8 6 8 7 7 7 6 7
4 4 6 6 6 6 0 3 7 7 7 7 6 6 6 6 7 6 6 7
6 4 6 3 7 3 0 6 5 4 7 6 7 3 8 7 0 7 6 7
2 6 6 7 4 7 6 7 4 6 7 6 6 7 7 6 8 7 3 6
6 7 7 7 7 7 8 6 7 6 6 6 0 7 7 8 5 7 4 6
6 5 7 7 7 6 6 7 7 7 7 5 7 7 7 7 7 6 6 7
7 5 6 7 7 6 4 3 7 6 6 7 7 6 6 7 6 6 7 7

BLOCK 7 - 4B

7 0 5 0 0 0 0 0 0 3 0 0 0 7 0 0 0 0 0 0
2 2 0 0 0 0 0 3 0 3 0 2 0 2 0 0 2 2 2 0
0 0 2 0 0 0 0 0 0 2 7 0 3 0 0 0 0 0 0 0
0 0 3 0 0 0 2 0 3 0 0 0 0 0 2 2 0 0 0 0
2 2 3 2 2 3 0 4 2 0 0 2 2 6 5 0 2 0 0 6
7 7 2 4 7 2 3 5 0 0 0 0 0 0 2 3 3 0 0 0
0 2 3 2 2 2 6 3 2 0 2 6 2 3 5 0 0 0 2 0
2 7 3 0 0 2 2 2 0 6 3 0 5 2 0 2 0 2 0 4
0 2 0 0 6 0 0 0 0 0 2 0 0 0 0 2 0 0 0 0 0
1 7 0 0 0 6 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0
0 0 6 0 2 0 0 0 3 0 0 0 0 0 0 0 0 0 2 0
2 0 0 2 0 0 3 0 0 0 0 0 0 0 0 0 0 0 0 2
0 0 5 3 7 0 2 7 0 4 0 0 2 0 0 6 0 0 7 1
0 8 0 0 0 0 0 0 6 0 0 7 0 0 0 0 1 0 0 7
3 0 0 0 0 0 0 5 0 7 0 2 0 0 5 0 0 6 7 0
3 0 0 0 0 3 0 0 0 5 2 0 0 0 3 3 7 0 0 0
0 0 0 2 0 0 0 3 0 0 3 0 0 0 0 0 0 0 0 0
2 0 0 2 3 0 0 0 0 2 0 3 2 0 2 0 7 0 3 2

BLOCK 8 - 2C

3 2 5 0 0 0 2 0 5 4 0 0 2 0 1 2 3 0 0 7
7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
7 7 7 7 6 7 7 7 6 6 6 6 7 6 7 6 6 7 7 7
6 2 6 6 2 7 7 7 5 7 7 7 7 7 7 6 6 7 2 0
8 7 7 7 8 7 6 7 7 7 6 6 6 6 8 7 8 2 0 7
7 7 7 8 7 7 6 7 7 6 7 6 7 7 7 7 8 7 7 7
0 2 0 3 0 4 3 0 4 6 5 5 3 0 5 6 4 3 0 3
7 5 7 6 7 7 7 7 7 7 7 5 5 2 6 6 5 7 6 7
6 7 7 7 7 7 0 0 7 7 7 1 7 7 6 6 8 0 7 7
0 0 7 7 0 2 0 0 0 1 0 0 0 0 0 7 0 0 6 0
3 7 7 7 7 6 6 4 7 7 6 7 7 6 8 7 6 5 6 6
6 3 5 6 7 6 5 6 7 7 6 6 0 1 0 0 7 3 6 6
3 4 7 6 6 6 6 6 7 0 6 2 7 6 8 6 6 7 6 6
7 7 7 7 7 7 6 6 7 7 7 7 7 7 7 7 7 5 5 7
7 7 7 7 7 7 6 7 7 7 6 6 3 6 6 6 6 6 6 7 7
7 0 6 6 3 6 7 6 6 7 7 7 6 6 6 6 7 0 6 5
7 7 2 7 7 3 6 7 4 0 6 3 4 2 6 7 7 6 6 7
3 7 7 7 2 3 0 7 0 2 6 7 0 0 7 7 5 6 2 6

BLOCK 9 - 2B

2 0 2 4 0 2 0 2 5 0 2 0 2 0 0 0 0 0 0 0
0 0 0 0 0 6 0 0 0 2 2 0 0 0 0 0 1 0 0 0
0 0 0 0 0 0 0 7 6 0 0 0 0 0 1 2 0 7 8 0
0 7 0 0 0 0 0 3 5 0 0 2 0 8 0 0 4 4 0 0
2 0 0 2 3 0 0 0 0 2 0 3 3 3 0 3 0 2 7 0
0 2 0 2 0 0 3 3 0 4 0 0 0 0 2 0 0 4 3 2
7 0 4 0 0 2 0 0 0 0 0 7 8 0 0 0 2 0 0 7
0 0 3 0 0 3 0 0 8 0 0 3 0 0 2 0 2 6 0 *
2 0 2 0 0 7 0 2 2 6 0 0 0 0 2 0 0 0 0 0
0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 2 0 2 0 0 0 0 0 3 7 0 0 2 0 0 2 0 2
5 0 6 0 2 0 0 0 0 0 0 0 2 0 0 2 0 0 2 0
0 0 2 2 0 0 0 0 2 7 0 7 0 0 0 0 0 0 2 0
3 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
6 2 7 0 7 7 7 7 7 6 0 2 7 7 6 6 0 2 7 5
0 0 0 2 0 0 2 0 0 0 2 2 2 7 0 0 2 2 0 0
0 0 0 7 0 0 0 0 2 0 3 0 6 7 0 7 0 0 7 0
6 0 0 2 0 0 0 0 0 6 0 0 0 2 0 2 0 0 7 0

BLOCK 10 - 4C

3 6 2 2 6 5 7 6 0 2 7 2 2 5 6 3 0 6 3 3
7 7 7 8 7 7 7 7 8 6 7 6 7 7 7 6 7 7 8 7
3 7 7 7 7 7 8 8 7 7 8 6 7 7 7 5 3 7 8 7
6 6 0 7 6 6 6 6 7 2 7 0 6 6 0 6 6 0 7 7
7 7 7 7 2 7 7 6 0 7 7 7 7 6 7 6 7 7 7 7
7 7 7 7 7 7 7 6 2 7 7 3 7 7 7 7 7 6 7 7 7
0 3 0 2 0 6 5 2 0 3 6 1 4 6 2 6 8 7 7 6
7 4 7 7 7 7 7 7 7 7 7 7 7 7 7 6 7 7 7 7 7
2 3 5 7 7 7 3 4 7 6 6 7 6 6 6 6 3 3 7 7
7 7 7 7 7 6 7 7 7 7 7 7 7 7 6 6 7 7 7 6
6 7 7 7 7 7 7 6 7 7 7 7 7 7 6 7 7 7 7 7
7 7 7 7 7 7 7 7 2 7 7 7 6 7 7 7 7 0 6 7
6 3 7 3 4 6 3 6 3 6 7 7 5 5 5 3 2 2 0 7
0 0 6 6 6 5 0 0 3 0 6 0 5 0 0 0 0 0 0 1
6 2 8 7 7 8 6 7 7 7 7 8 7 7 7 6 7 7 7 7
0 2 2 3 7 1 6 0 0 4 3 0 0 6 7 6 7 4 0 0
6 3 7 7 7 7 7 7 7 7 7 7 7 7 6 6 7 7 7 7 7
7 7 7 7 7 7 7 7 5 7 6 7 7 7 7 4 7 7 6 6

BLOCK 11 - 1A

7 6 7 7 6 5 7 7 6 7 6 6 6 6 6 6 7 7 7 6
7 6 7 6 7 6 7 7 6 6 7 7 7 6 6 6 7 7 7 6
2 2 7 6 6 7 7 7 7 7 6 6 7 7 6 6 7 7 7 7
6 5 7 7 7 7 7 7 6 5 7 7 7 7 7 7 7 5 7 7
6 6 0 7 0 6 5 2 7 6 6 0 7 7 7 6 2 0 6 6
7 6 7 3 7 6 5 6 7 4 6 5 6 7 0 7 0 7 2 4
7 7 8 7 7 7 7 7 6 8 7 7 7 7 8 7 7 7 7 7
7 7 0 0 7 0 4 0 0 0 1 0 0 3 1 0 0 0 1 0
2 6 6 5 5 6 7 6 6 4 6 5 6 4 6 3 7 6 6 6
8 7 6 7 6 8 6 7 7 7 6 6 6 8 8 8 7 7 7 7
8 7 6 7 7 6 7 7 7 7 7 7 7 7 7 7 7 7 8 7
7 7 6 5 6 4 2 6 7 7 7 6 7 7 7 7 7 7 6 5
6 7 7 7 7 7 8 6 7 7 7 7 7 7 7 7 7 7 7 7
6 6 7 7 7 6 6 6 6 7 7 6 6 7 7 7 6 7 6 6
0 0 6 3 0 7 0 7 7 7 1 0 7 7 7 2 7 0 7 2
5 6 3 6 3 2 6 6 2 7 2 6 3 2 3 4 3 5 3 6
6 5 4 7 6 6 6 7 7 6 6 6 7 7 7 7 6 7 7 5
7 7 7 7 7 7 7 7 7 7 7 7 7 6 7 7 6 7 7 7 7

BLOCK 12 - 3B

0 0 6 6 0 5 4 7 2 0 0 0 0 0 3 0 0 2 0 6
6 6 2 2 0 7 7 7 7 6 6 6 6 6 6 7 6 6 6 7
7 6 6 6 7 7 5 7 7 7 7 7 5 7 7 7 7 7 7 6
6 7 7 4 2 4 7 7 7 6 7 6 6 7 2 0 7 7 0 7
7 7 7 7 7 7 6 7 7 7 7 6 6 6 6 7 6 7 6 7
4 6 7 6 7 7 7 7 7 7 6 7 7 6 7 7 7 7 7 7
3 4 7 7 4 3 6 0 0 6 6 4 5 6 7 7 3 4 7 3
7 7 6 7 6 2 6 6 7 7 6 7 7 7 6 6 5 6 6 6
3 2 6 3 6 6 6 7 6 5 0 7 6 6 3 7 0 0 7 2
7 6 6 3 6 7 2 4 6 4 6 5 6 5 7 3 5 3 3 7
7 7 7 7 7 7 7 7 7 7 7 7 7 6 7 7 7 7 7 7
7 3 7 7 7 3 7 7 7 6 7 7 2 7 7 7 2 7 7 7
6 6 7 7 7 6 4 7 7 7 6 5 7 6 6 6 7 7 7 7
4 6 7 7 5 0 4 0 6 6 0 7 6 0 3 4 0 0 0 0
7 7 7 3 6 2 2 6 6 3 0 7 4 5 2 0 6 5 2 7
7 7 7 7 6 7 8 7 7 7 6 7 7 7 7 7 7 7 8 7
7 7 7 7 7 6 6 7 7 7 7 7 7 6 7 7 7 4 7 7
3 6 6 7 7 7 7 7 7 0 7 7 7 7 7 3 7 7 7 7

BLOCK 13 - 4A

2 2 0 0 0 0 7 0 2 0 0 0 0 0 0 0 2 0 6 0 0
7 7 7 6 7 7 6 6 7 7 7 7 7 7 7 7 6 7 6 7
6 6 6 6 7 0 6 6 7 6 7 6 6 7 6 6 2 7 6 6
6 6 6 7 6 0 6 6 7 6 2 7 6 6 6 6 7 7 7 7
6 7 7 7 7 4 7 2 7 7 0 7 7 6 6 7 7 6 7 0
7 6 7 2 7 7 7 6 6 6 6 6 6 6 8 6 6 6 7 7
4 3 2 3 6 0 6 0 4 3 0 5 3 4 6 0 3 5 0 4
7 7 6 6 2 3 7 7 7 7 8 7 7 7 7 7 8 7 7 7
7 7 7 7 7 7 7 7 7 7 7 7 7 7 6 6 3 0 7 7
7 7 3 7 7 6 6 6 6 0 6 0 4 0 0 7 6 0 0 2
7 3 2 2 3 7 0 7 2 7 7 0 4 0 7 6 6 6 7 7
2 6 5 7 3 7 2 2 6 6 7 4 7 6 3 7 2 7 0 0
6 0 0 6 6 6 6 0 0 5 0 5 4 7 7 3 0 0 0 3
7 7 7 7 7 7 6 5 7 6 7 7 6 6 6 7 7 6 7 6
7 7 2 6 5 3 7 6 6 7 7 6 7 7 0 0 7 6 6 7
2 6 7 7 7 6 7 6 0 6 6 3 6 6 8 4 6 6 6 6
6 6 3 6 6 8 0 3 7 7 7 8 7 7 7 0 5 6 7 7
2 6 7 7 7 6 5 7 4 6 7 3 6 6 7 6 7 6 7 7

BLOCK 14 - 2B

0 0 0 3 4 2 0 0 0 0 6 0 0 0 0 0 2 0 0 0
0 0 0 6 0 0 0 0 0 2 0 0 3 0 2 2 7 7 3 2
0 7 0 3 3 2 4 0 0 0 0 0 7 3 0 0 0 0 0 2
0 0 0 0 0 0 2 0 5 0 2 0 0 2 0 0 0 0 2 2
0 6 6 0 6 0 0 0 0 0 0 4 0 0 0 3 0 4 7 2
2 5 3 2 3 2 6 2 0 0 2 2 2 2 2 0 2 0 0 0
4 7 0 2 3 0 0 0 2 0 2 2 0 0 3 0 0 5 0 0
0 0 3 0 0 2 6 0 0 0 3 5 5 0 3 6 0 0 0 0
2 6 0 0 6 5 0 0 7 0 0 7 2 6 0 2 0 0 3 7
2 0 3 2 3 0 0 0 3 2 3 0 0 0 7 7 0 0 0 2
0 0 0 0 0 0 0 0 7 0 0 0 2 0 0 3 7 0 7 0
5 0 2 2 0 0 0 0 2 0 2 0 0 2 4 5 0 0 0 2
0 7 0 7 0 0 0 0 5 0 0 0 0 0 0 0 4 7 0 0
0 0 0 3 7 0 0 0 2 7 7 1 0 0 0 7 0 0 0 0
0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 2 0 0 2 7 0
2 2 2 2 3 2 6 0 2 3 0 0 2 2 2 2 0 3 2 0
0 0 0 2 0 0 0 0 0 0 3 0 0 0 0 0 0 0 2 0
0 2 0 0 0 2 0 7 0 0 2 0 2 3 0 0 0 0 2 0

BLOCK 15 - 3C

2 6 2 6 0 2 6 5 2 6 2 2 3 4 6 7 2 5 6 2
7 7 7 7 7 7 6 7 6 7 7 7 6 7 8 8 7 8 7 7
6 7 7 7 7 8 6 6 5 5 7 7 7 7 7 6 7 7 7 7
4 3 4 5 6 2 6 6 2 7 7 6 0 4 6 7 7 6 7 3
7 7 7 7 7 6 7 7 7 7 6 7 7 7 7 7 7 7 6 7
7 7 7 7 7 7 7 6 7 7 7 6 6 6 7 7 7 7 7 7
0 6 6 6 6 7 6 3 5 6 6 6 7 3 5 2 6 7 6 0
7 7 7 7 7 7 7 7 6 7 7 7 6 7 6 7 7 7 7 7
2 2 3 3 0 6 2 3 2 6 5 6 0 7 0 3 2 6 7 6
7 7 6 7 7 7 7 7 7 7 7 6 6 7 7 5 5 7 7 6
8 7 7 7 7 8 7 7 7 6 8 7 6 7 7 7 7 7 8 7
7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 6 6 7 6
7 4 6 7 6 6 7 7 5 6 7 7 7 7 7 6 7 2 6 7
2 6 0 6 6 0 0 6 0 0 0 0 0 2 0 0 1 1 1 0
7 7 7 0 0 7 7 0 7 4 3 2 7 5 7 8 2 6 6 6
7 7 1 0 0 6 0 0 0 2 7 0 6 1 6 7 0 7 2 0
7 7 7 7 7 7 7 7 7 7 7 7 7 8 6 7 7 7 7 7
6 5 6 6 6 7 7 7 6 7 5 6 6 6 7 7 7 6 7 6

TABLE A4.3 GROWTH ROOM TRIAL - WHEAT AND TRITICALE

REPLICATE 1

BLOCK 1 - CL/C

0 0 0 0 0 0 0 * 0 0 0 0
0 0 0 0 0 * 0 * 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
* 0 * 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 * 0 0 0 0
0 * 0 0 0 0 0 0 * 0 0 0
* 0 * 0 0 0 * 0 * 0 0 *
0 0 0 0 0 * 0 0 0 0 0 0

BLOCK 2 - SF/A4

1 1 1.5 1 1 1 * * 1 1 * 2
2 1 1 2 * 1.5 * 1 1 1.5 * 2
2 2 1.5 2 1 1 1 2 1 2 1 1
0 * * * 1 * * 0 * 1 1 2
* * * 2.5 * * 2 1 0 1.5 0 1
1.5 2 1.5 1 2 1.5 2 1.5 2 2 2.5 *
1 2 2 1 1 2 1 2 1 1 2 2.5
1 1 1.5 * 2 * * 1 * 1 0 2.5
2.5 0 0 3 3 2 1 1.5 4 0 2 3

BLOCK 3 - FE/B3

2 3 2 1 0 2 * 2 0 1 3 2
1 * 1 2 1 1 1 1.5 2 1.5 1.5 2
4 4 * * 4 4 0 5 4 4 4 2
0 0 2 1 * 1 1 1 1 * 1 0
* 1 4 1 2.5 5 5 5 5 5 2 *
2 1.5 2 2 2 2 1.5 2 2 * * 1
2 2 1.5 2 * 2.5 1 * 1.5 * 2 1.5
4 3 3 * 2 1.5 3.5 3 0 * * 4
5 3 * 5 * 5 * 4 0 5 5 4

REPLICATE 2

BLOCK 4 - FE/B4

1 1.5 3 2 2 1.5 1 2 2 2 2 2
2 2 1 1.5 1 1 2 1 1.5 1 1 2
4 4 4 2.5 2 5 4 2.5 4 5 4 4
1 * 1 1 0 2 1 1 * 1 1 1
5 5 * 1 4 5 2.5 4 4 5 5 5
2 5 2 2.5 2 2 2.5 2 2 2 2 2
* 1.5 2 2 1 1 1.5 2 1.5 * 1.5 2
* * 3 2 2 3 5 * 3 2 4 *
* * 2.5 2 4 4 * * 1 * * 2

BLOCK 5 - CL/A

0 0 0 * 0 0 0 0 0 0 0 0
0 0 * * 0 0 * 0 0 0 0 0
0 0 * 0 0 0 * * 0 0 0 0
* 0 * 0 0 0 * * 0 0 0 0
* 0 * 0 0 * * * 0 0 0 0
0 0 0 0 0 0 * 0 0 0 0 0
0 * 0 0 0 0 0 0 * * 0 0
* * * 0 0 0 * 0 0 0 0 *
0 0 0 0 0 0 * 0 0 0 0 0

BLOCK 6 - SF/C2

* 2 2 *
2 1.5 1.5 2.5
1 2 2 1
2 2 3 0
* 1.5 1.5 1
2 0 2 1
1 0 * 1
* 1 2 1.5
1.5 2 2 1.5

2 1.5 1.5 2
* 1 * 1
2 1 1 *
1 0 2 2
1 2 1 1
2 * 2 2
2 1 3 4
1.5 1.5 1.5 1
1 1 * 1

1.5 2 * 2
2 1.5 1 1
* 1 1.5 2
1 * 1 2
1.5 2 2 1
1 * * 2
1 1 3 *
1.5 1 2 1
* 2 * *

REPLICATE 3

BLOCK 7 - SF/B1

2 1 2 2
1 0 1.5 1
1.5 5 5 1
1 2 * 2
1 2 3.5 3
1 1 1 1
1 1 1.5 1
3 1.5 1 1.5
* 0 4 2.5

1.5 * 2 1
1 2 1 1.5
2 0 4 2
1 1 1 *
1 0 3.5 3
2 0 1 1
0 1 2 *
* * * 1
0 0 2 2.5

1.5 2 * 0
2 2 1 1
2 1 2 3
1 2 * 1
0 2 2 0
1 * * *
1 2 * *
* 2.5 2.5 1
* 5 * 1

BLOCK 8 - FE/A2

1 1.5 1 2
1 1 2 1
* * 2 3
4 3 4 2
4 2 * 2
2 2.5 2 2
4 4 2.5 2.5
4 5 4 4
2 4 * 4

1 1 0 *
1 0 0 1
2 * 1 *
3 2 2 2
5 4 4 3.5
2 2 2 2
4 3 3 2.5
3.5 4 5 5
4 4 3 *

0 1 2 1
1 1 * 1
2 * 2 1.5
2 * 2 2.5
4 4 4 4
2 3 3 1
4 3 * 4
* 5 * 4
5 5 5 3

BLOCK 9 - CL/C

```
* 1 * 0 4 * * 0 1 * 0 0
* 0 * 0 1 1 0 0 0 * 0 2
1 1 2 0 0 1 * 1 0 1 0 2
0 0 0 0 0 * 0 0 0 1 0 1
0 0 0 0 0 * 0 0 0 0 0 0
0 0 0 0 0 0 * 0 0 * 0 0
* 0 0 0 * * * 4 * 2 2 0
0 * 0 0 * 0 * 0 0 0 0 0
* 0 0 0 0 * * 2 2 3 0 0
```

REPLICATE 4

BLOCK 10 - SF/B3

```
2 3 1 3          1 * 1 1        2 1 2 1
1 2 1 2          1 2 1.5 2      * 1.5 1 1
2 * 1.5 2        3 1.5 * 2      1 2 3.5 4
* 1 2 1.5        2 1 1 1        * 1 1.5 1
1 3 1.5 1        2 1.5 * 2      3 2.5 1 2
1 1 * 1          1.5 * 1 0      1 1 * 0
2 1.5 * *        1 * 2 1        1 1 1.5 *
2 * * 2          * * * 1        1 0 * 1
1 * * *          3 0 1.5 2.5    1 * 2 2
```

BLOCK 11 - FE/A1

```
2 2 0 1          2 * 2 1        * 2 2 2
* 0 2 1          1 0 0 2        0 1 1 *
3 2 2.5 2        2 3 1 2.5      2 2.5 3.5 2.5
2 2.5 2.5 4      3 2.5 2 2.5    4 4 2 2
5 * 4 1.5        1.5 4 4 3      5 4 4 *
1 2 0 2          3 3 2 *        2 2 4 2
* 2.5 4 5        4 1 * *        3 * 3 3
5 * * *          * 3 * 4        * * * 5
* * 5 5          * * * 2.5      * 2 * 5
```

BLOCK 12 - CL/C

```
0 * 0 0 0 0 * 0 * 0 0 0
0 * 0 * 0 0 * * * * 0 *
0 0 0 0 * 0 0 0 0 * 0 0
0 0 0 0 0 0 * 0 0 0 0 *
0 * 0 0 * 0 * * 0 0 0 0
0 * 0 0 0 0 0 * 0 0 0 0
0 0 0 * 0 0 0 0 0 0 0 0
* 0 0 * * * 0 0 0 0 * 0
0 * 0 0 0 0 * 0 0 0 0 0
```

TABLE A4.4 CULTIVAR GROWTH ROOM TRIAL - BARLEY AND RYE

REPLICATE 1

BLOCK 1 - CL/C3

0 0 0 0 0 0 0 1 0 0 0 0
* 0 1 1 0 0 * 0 0 0 * *
0 * 0 0 0 0 0 0 0 0 0 0
* 2 * * 0 0 0 0 0 * 0 0
* * * 0 0 0 0 * * 0 0 0
0 * 0 0 0 0 0 0 0 0 0 0
0 0 * * 0 0 * * * * * *
0 0 0 0 0 0 0 0 0 * 0 0

BLOCK 2 - FE/B4

4 1.5 1 1 1 3.5 2 4 * 1 1.5 1
2 1 2 2 * 4 2 * 2 2 4 0
* * 1 1 * 1 3 * 1 1 0 1
1 1 * 1 * 2 4 2 2 2.5 1 2.5
1.5 1 1 0 2 1 * 1 1.5 1 1 *
1 4 * * 3 1.5 1 2 2 * 1 *
1 2 3 2 2 2.5 2 * 1 1 1 1.5
* * 0 1 1 * 0 * 1 0 0 1

BLOCK 3 - SF/A3

1 1 2 1 1 2 1 1 1 * 1 2
2 1 2 1 1 1 * 0 * 0 0 1
0 * 1 * * * 1 3 * 2 1 0
2 1.5 2 2 * * 2 1.5 1 0 * 1
1 1.5 1 * * 1 1 0 1.5 2 1 2
0 2 0 2 0 1.5 1 * 0 0 2 2
* * * * * * * * * * * *
* * * * * * * * * * * *

REPLICATE 2

BLOCK 4 - SF/B2

3 1 4 2 2 2 * 2 1 2 2 1
2 1.5 2 3 4 3 * 2 4 2 2.5 *
0 2 1 * 2 1.5 * 1 * 2 * *
2 3 2 2 * 2 * 2 2 1.5 * 2
1 2 * 1 1 1 1 2 2 3 2 *
* 2 * 3 2.5 * 1 2 2 2 2 1
2 4 4 * 2 2 * 3 5 3 2 2
* * * * * * * 0 * * 0 *

BLOCK 5 - CL/C

0 0 1 0 1 0 0 * 0 0 0 0
0 0 1 2 1 1 0 * 2 1 1 1
1 * 2 1 1 0 1 2 1 1 0 1
1 * 1 0 1 1 1 1 2 0 0 1
* * * * * * * * * * * *
0 0 0 0 0 1 0 0 0 0 0 1
0 0 0 0 0 0 * * * 1 0 1
0 * 0 0 0 0 0 * 0 0 0 0

BLOCK 6 - FE/A2

2 3 4 1 4 3 2.5 2 2 2 2 2
1 * 1 2 2 2 2 1 0 2 0 3
1 0 0 1 1 1 * 0 0 0 1 1
2 2 0 2 1 2 1 1 2 * 1 2
1 0 1 1 0 1 * 1 1 0 1 0
2 3 1 * 1 * * 1 * * * *
2 * 4 3 0 1 2 * 1 4 1 *
* 1.5 * * 2 * 2 1 * 1 4 1

REPLICATE 4

BLOCK 7 - CL/B

* 0 * 1 0 0 0 0 0 0 0 0
0 * 0 0 0 0 0 * * 1 0 0
* * * 0 * 0 0 0 * * 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 0 * 0 0 0 0 0 * 0 0 *
0 * 0 0 0 0 * 0 * 0 0 0
1 0 0 0 0 0 0 0 0 0 0 0
* * * * * * * * * * * *

BLOCK 8 - FE/A3

* 5 4 1 5 1 1 1 2 2 1 1
2 5 0 0 1 * 1 1 1 1 2 *
0 * 0 1 1 0 1 * 1 0 2 0
1 1 1 2 2 * 2 2 1 2 2 1
1.5 1 0 0 1 1 0 * 0 1 0 0
2 2 1 2 1 1.5 4 1 4 2 * 1
2 * 1 * 2 2 1.5 1 1 * * 1.5
* 1 * * * * * * 1 1 1 *

BLOCK 9 - SF/C1

* 0 1 0 1 0 0 0 * 0 0 0
2 * 1.5 1 1 * 1 0 1 1 1 2
2 2 2 2 1 1 1 1.5 2 0 * *
0 1 2 2 2 2 * 2 1 2 2 1
* * * * * * * * * * * *
* * * * * * * * * * * *
* * * * * * * * * * * *
* * * * * * * * * * * *

REPLICATE 4

BLOCK 10 - FE/A1

4 2 1 *	4 * 3 4	4 2 1 1
3 * 2 1	1 3.5 2.5 4	2 1 1 1
0 1 1 1	1.5 0 0 0	0 * 1 1
2 2 2 *	3 5 1 2	1 2 2 1
1.5 * * *	* 1.5 1.5 2	2 1.5 1.5 *
1.5 5 0 1.5	2 * 0 *	1.5 * * *
2 2 2 4	2 2 1 3	2 0 5 4
4 2 * 2	* * * *	2 1 1 1

BLOCK 11 - SF/C4

1 1 2 1	2 1 1 1	1 2 1 1
1 2 2 1.5	0 1 1 2	* 1 2 2
2 2 2.5 2	2 2 2 0	1 2 1.5 2
1.5 4 1.5 2	2 1.5 2 2	* 1 1 2
1.5 2 2 1.5	* 1 * 1	* * 1.5 *
* 2 2 3	* 1.5 * *	* 1 1 2
2 1 * 1	1 * 2 *	1.5 1 0 2
0 1 * 0	0 1 0 2	* 0 2 0

BLOCK 12 - CL/B

0 0 0 0 0 1 * * * * * *
* * * * * * * 0 0 * 0 0
0 * 0 * 0 0 0 0 * 0 0 0
0 * 0 1 0 0 0 * * 0 * *
0 * 0 0 0 * 1 0 0 * * *
0 * 0 0 2 1 0 0 1 2 1 1
1 * 0 2 1 2 1 2 1 1 1 0
* * * * * * * * * * * *

TABLE A4.5 GROWTH ROOM CULTIVAR TRIAL - SLOW-FEATHERY ONLY, WHEAT AND TRITICALE

BLOCK 1

1 2 * 1	1 1 1.5 2	2 1 0 2
0 1 3 2	2 * 1 *	1.5 1 1 2
* 2 1 2	4 4 * 2	1.5 1 2 2.5
* 2 3 1	2 1 2 2	2 1 1 2
2 * 2 1	0 2 1 2	2 2 1 1
1 1 2 1	2 * 2 1	1.5 1 1 2
2 2 1 0	* 1.5 2 1	* 2 1 1
1 1 * 1	1 2 2 *	2 * * *
* 2 * 2	* 3 2 *	2 * 2 2

BLOCK 2

1 2 2 2	1 1 2 1	* 2 3 1
1.5 2 * *	0 * 2 2	1 1 * 1
1 2 1 1	* * 2 1	2 2 3 2
* * 2.5 2	* 1 1 *	1 2 1 1
0 2 1 1	0 2 1 2	1.5 2 0 0
* 2 1 2	1 2 * 1	3 4 2 2
1 1 * 1	1 1 0 1	1 * 1 2
* 1 3 1	2 * 1 1	2 * 2 2
* * 2 3	3 0 3 3	1 1 2 2

BLOCK 3

1 1 * 2	2 2 1 2	3 * * 2
1 * 2 2	1 1 1 1	1 2 2 2
1 1 * 3	1 2 2 2	1 2 2 1
* * 0 0	4 2 2 1	2 0 * 2
2 0 1 *	1 1.5 1 2	1 2 1 1
* 0 0 *	3 0 * *	1 0 * 2
* 0 0 4	* 4 3 3	0 * 1 2
* 1 1 1	1 1 2 1	1.5 2 * 1
1 * 1 1	1 1 * 1	* * * 1

BLOCK 4

2 2 * *	* * * *	* * * *
* 0 * 2	* * 2 0	* * 2 1
0 2 2 1	2 2.5 * 2	1 2 2 2
5 1 2.5 2	1 * 2 2	2.5 2 * 4
5 1 4 5	2 5 4 3	4 4 4 4
1 2 1 4	* 2 * 3	3 * 5 4
* 4.5 4 3	3 4 1 2	5 4 4 *
3 * 1 1	5 4 2 2	* 3 4 *
2 2 3 2	2.5 * * *	* * * 1

TABLE A4.6 GROWTH ROOM CULTIVAR TRIAL - SLOW-FEATHERY ONLY. BARLEY AND RYE

GROWTH ROOM TRIAL - BARLEY/RYE SLOW FEATHERY TREATMENT ONLY

BLOCK 1

1 1 1 *	1 1 1 *	2 1 2 3
* 1.5 2 *	* 1.5 1 *	2 * * 2
2 5 2 1	2 5 1 2	1 5 5 3
1 5 1 5	1 1 1.5 1	1 2 3 2
* 5 3 *	* 4 * *	* 4.5 1 5
2 2 1 2	2.5 1 1 2	* 1 1 2
1 0 * *	1 1 2.5 5	5 2 1 1.5
4 1 2 2	* 2 1 0	5 3 5 1.5

BLOCK 2

2.5 1 2 4	2 * * 2	2.5 2 5 2
2 2 4 1.5	1 1 1 1	* 2 1 1
2 2 3 0	0 1 0 *	2 5 3 2
1 2 * 1	5 2 1 0	0 2 * 2
* 1 0 0	0 * * *	0 0 * 2
2 1.5 2 *	* 1.5 2 4	2 1 2.5 1.5
* 2 5 5	1 5 2 2	* 5 2.5 5
1.5 * * *	* * * 2	2 * * *

BLOCK 3

2 4 5 1.5	5 1.5 5 4	1 1 1 3
2.5 2 1.5 1.5	2 1 * *	1.5 2 2.5 1
2 0 0 *	* 3 1.5 2	1.5 2 3 2
* 1.5 2 *	4 * 5 0	* * 1 2
1 1 1.5 1	4.5 1 1 1	* 2 2 4.5
4 5 1.5 1	2 5 3 5	4 5 1 1.5
2 2 2 3	2 2 2 5	4 3 2 4
* * 3 3	* * 0 *	* * * *

BLOCK 4

2 5 3 4	4 5 2 2	5 2 2 *
5 4 5 3	5 2 5 4	4 3 2 2
3 5 1.5 5	2 0 2.5 2	4 3 2 4
1 5 * 4	* * 5 2	2.5 2.5 2 *
0 0 2.5 1	0 0 2 *	1 2.5 2 0
2 5 2 2	5 4 5 5	1 1 * 2.5
2 1 1.5 2	1.5 4 2 0	4 2 1.5 2.5
* 2 * *	3 0 * *	* 2 * *

TABLE A4.7 WHEAT LINES - Growth room results

A,B and C = slow-feathery isolates
D,E and F = fast-even isolates

Line	Scores Pot 1	2	3	4
18	A 1	C 2	E 2	
	A 0	C 2	F 1	
	B 1	D 2.5	F 1	
	B 1	D 2	F 2	

Means

SF 1.17 std dev. 0.75
FE 1.75 std dev. 0.612

19	Pot 1	2	3	4
	A 0	A 0	B 1	C 1
	A 0	A 0	B 2	C 3
		B 0	C 2	E 5
		B 0		E 2.5

	Pot 5	6	7	8
	C 1	E 4	E 3	Control 0
	D 2	E 2	E 5	" 0
	D 4	F 1	E 3	" 0
		F 3	E 3	" 0

Means

SF 0.83 std dev. 1.029
FE 3.125 std dev. 1.208

20	Pot 1
	B 1
	C 2
	E 5

Means

SF 1.50 std dev. 0.707
FE 5.00

21	Pot 1	2
	A 0	A 2
	B 1	C 2
		D 2
		D 2

Means

SF 1.25 std dev. 0.957
FE 2.0

22	Pot 1	2	3	4

A 0	B 1	C 2	D 1
A 1	B 2	C 2	D 2
A 2	B 2	C 2	D 2
A 2	B 2.5	C 1	D 2

Pot 5	6	7	
E 1	F 2	Control	0
E 2	F 2	"	0
E 2	F 2.5		
E 2.5	F 2.5		

Means

SF 1.625 std dev. 0.71
FE 1.958 std dev. 0.498

23

Pot 1	2	3	4
A 0	B 1.5	C 1	D 5
A 0	B 3	C 2	D 5
A 2	B 4	C 2	D 2
	B 5	C 5	D 5

5	6	7	
E 5	F 3	Control	0
E 2	F 4	"	0
E 5	F 4	"	0
E 5	F 5	"	0

Means

SF 2.318 std dev. 1.765
FE 4.16 std dev. 1.194

24

Pot 1	2	3	4
A 1	B 1	C 0	D 2
A 1	B 1	C 2	D 2
A 1	B 2	C 2	D 2
A 2	B 2		D 2

Pot 5	6	7		8
E 1	F -	Control	0	F 1
E 2	F -	"	0	F 1
E 3	F 1	"	0	
	F 2	"	0	

Means

SF 1.364 std dev. 0.674
FE 1.727 std dev. 0.647

25

Pot 1	2	3	4
A 0	B 1	C 2.5	D 2
A 0	B 1	D 2.5	D 2.5

A 1	C 1	E 3
B 2	C 1	E 2.5

Pot 5	6
A 2	Control 0
F 2	" 0
F 1	

Means

SF 1.15 std dev. 0.818
FE 2.214 std dev. 0.636

26

Pot 1	2	3	4
A 4	B 1	D 5	E 5
A 0	B 5	D 5	E 5
B 4	C 5	E 5	F 5
	C 5		F 5

Pot 5
Control 0

Means

SF 3.429 std dev. 2.07
FE 5.0 std dev. 0

27

Pot 1	2	3	4
A 0	B 1	E 2.5	F 1
A 0	B 1.5	E 2	F 1
A 1	B 2	E 2	F 0
A 1	B 2	E 2	F 1

Pot 5	6	7	8
Control 0	Control 0	C 2	D 2
" 0	" 0	C 1	D 2
" 0	" 0	C 2	D 2
" 0	" 0	C 2	D 2
		C 1	

Means

SF 1.269 std dev. 0.725
FE 1.625 std dec. 0.711

28 Pot 1

A 0

Mean = 1

29 Pot 1

A	1
B	3
E	1

Means

SF 2 std dev. 1.41
FE 1 std dev. E

30 Pot 1 2

A	1	D	2
B	2		

Means

SF 1.5 std dev. 0.707
FE 2.0 std dev. E

31 Pot 1

A	1
B	1

Mean = 1

32 Pot 1 2 3

A	1	C	2	E	1.5
A	1	D	2.5	E	1.5
B	3	D	3	E	2
B	2.5	F	2	E	2

Means

SF 1.9 std dev. 0.894
FE 2.07 std dev. 0.535

33 Pot 1 2

B	1	D	2
C	1	D	2
C	2	E	4

Means

SF 1.33 std dev. 0.577
FE 2.66 std dev. 1.155

34 Pot 1 2

A	2	E	5
A	0	F	5
B	3	F	4

Means

SF 1.66 std dev. 1.527
FE 4.66 std dev. 0.577

APPENDIX 5 - BARLEY TRIAL SCORES

TABLE A5.1 BARLEY YIELD TRIAL SCORES

REPLICATE 1

SAMPLE	INF.	HNO.	M.WT/H	NO.GR	GRWT
S3a	5	69	1.1391	1939	67
S3b	4	26	1.1192	710	23.9
S3c	4	53	1.1000	1405	48.7
S3d	4	73	1.1027	1926	66.8
S2a	3	50	1.132	1291	45.7
S2b	4	70	0.9771	1727	57.8
S2c	5	85	1.1047	2227	77.66
S2d	3	63	1.1513	1759	58.5
S4a	3	65	1.120	1597	58
S4b	1	44	1.0929	1104	37.97
S4c	1	66	1.2273	1822	67.89
S4d	3	39	1.1436	1075	38.98
S1a	4	93	1.2303	2508	92.56
S1b	4	76	1.0516	1922	63.5
S1c	4	82	1.2705	2281	85.68
S1d	3	53	1.1509	1414	49.2
F2a	6	51	1.0608	1224	45.3
F2b	4	86	1.1488	2339	82.7
F2c	5	58	1.0241	1535	50.19
F2d	5	122	1.1349	3197	107.8
F3a	*	74	1.1419	1922	69.2
F3b	5	48	1.2229	1331	50.01
F3c	4	75	1.1439	1994	69.3
F3d	5	85	1.1871	2253	78.6
F1a	5	84	1.6667	2171	81.9
F1b	4	114	1.2026	1282	47.6
F1c	3	103	1.2274	2968	116.4
F1d	4	105	1.3057	2922	114.3
F4a	3	101	1.2564	2672	104.8
F4b	3	105	1.2583	2811	106.4
F4c	2	110	1.2600	3174	119.6
F4d	3	86	1.1965	2347	85.2

INF. = INFECTION SCORE

HNO. = NUMBER OF HEADS

M.WT/H = MEAN WEIGHT PER HEAD

NO.GR = NUMBER OF GRAIN PER SAMPLE

GRWT = WEIGHT OF GRAIN PER SAMPLE

* DENOTES MISSING VALUE

S = SF
F = FE

1 = 10g / m2
2 = 20g / m2
3 = 30g /m2
4 = control

a-d = within-plot samples

REPLICATE 2

F4a	1	116	1.3179	3290	127.3
F4b	1	170	1.2595	4772	180.82
F4c	5	81	1.1778	2164	79.3
F4d	5	65	1.3207	2795	107.6
F2a	6	84	1.1524	2231	81.3
F2b	5	112	1.0741	2924	100.5
F2c	*	*	*	*	*
F2d	5	118	1.1754	3211	114.4
F3a	4	82	1.1555	2374	78.9
F3b	4	115	1.2015	3081	111.4
F3c	5	75	1.1969	2110	72.36
F3d	5	*	*	*	*
F1a	4	82	1.1437	2173	78.65
F1b	4	108	1.2352	2960	112.6
F1c	4	57	1.1754	1486	56.5
F1d	4	71	1.2218	1992	72.01
S4a	4	74	1.2297	2116	78.15
S4b	3	68	1.2544	1876	72.2

S4c	3	72	1.1571	1936	70.0
S4d	2	97	1.2567	2630	101.5
S3a	3	76	0.7724	2261	83.59
S3b	3	80	0.7583	2237	83.33
S3c	4	72	*	1894	67.5
S3d	3	63	1.2519	1662	64.2
S1a	5	121	1.1691	3234	113.5
S1b	3	73	1.1980	1927	72.5
S1c	4	51	1.2255	1352	52.3
S1d	5	69	1.3899	2027	81.3
S2a	2	73	1.2356	1850	67.82
S2b	2	113	1.2713	3171	120.0
S2c	1	70	1.2068	1907	70
S2d	3	87	1.2291	2440	87.7

REPLICATE 3

F1a	5	136	1.1343	3573	125
F1b	4	96	1.1417	2495	92.1
F1c	5	137	1.1190	3473	130.1
F1d	3	82	1.1329	2184	77.8
F4a	5	80	1.2151	2126	78.19
F4b	1	90	1.1756	2396	89.4
F4c	3	115	1.1400	3004	110.9
F4d	1	127	1.1787	3154	123.2
F2a	5	64	1.1500	1762	61.7
F2b	5	92	1.1598	2465	87.2
F2c	6	76	1.1105	1894	68.3
F2d	3	79	*	2082	80.2
F3a	6	54	1.0593	1426	49.52
F3b	4	108	1.0694	2871	96.3
F3c	5	64	0.9719	1481	50.48
F3d	4	86	0.7037	2116	77.54
S3a	5	64	1.1539	1598	59.17
S3b	5	79	1.0285	1924	67.01
S3c	5	60	1.1617	1591	58.3
S3d	*	85	1.1024	2209	80.2
S2a	5	75	1.2453	2003	76.8
S2b	3	75	1.1841	1843	67.6
S2c	5	51	1.0890	3064	116.5
S2d	4	76	1.2222	2005	75.08
S1a	6	53	1.2283	1325	54.3
S1b	5	39	1.0821	952	34.87
S1c	4	76	1.2247	2000	80.0
S1d	4	68	1.2875	1904	72.2
S4a	2	52	1.2462	1400	54.7
S4b	1	69	1.0876	1816	70.09
S4c	1	53	1.0424	1508	57.6
S4d	1	66	1.2546	1785	70.1

REPLICATE 4

S2a	3	161	1.2224	4085	154.3
S2b	4	93	1.1132	2284	85.1
S2c	4	44	*	1179	46.45
S2d	3	58	1.3175	1439	59.5
S4a	3	40	1.3203	1031	42.36
S4b	1	70	1.2047	1812	68.72
S4c	3	81	1.3691	2245	92.2
S4d	*	*	*	*	*
S3a	4	52	1.1981	1322	54

S3b	3	83	1.1723	2234	82.2
S3c	4	75	1.3027	2106	82.7
S3d	4	79	1.2390	2088	80.26
S1a	3	49	1.2612	1331	52.1
S1b	4	53	1.3774	1471	58.6
S1c	4	70	1.2171	1847	70.1
S1d	5	140	1.1449	2454	95.06
F4a	3	82	1.2700	2286	34.57
F4b	4	84	1.2929	2331	91.0
F4c	5	49	1.1539	1303	49.04
F4d	2	63	1.3078	1688	69.16
F1a	6	64	1.2364	1711	63.79
F1b	4	96	1.1460	2576	91.1
F1c	5	71	1.1616	2081	80.9
F1d	5	94	1.2261	2513	92.9
F3a	4	104	1.3173	2879	112.3
F3b	5	78	1.2051	2199	79.5
F3c	5	114	1.1623	3069	112.9
F3d	3	91	1.1507	2306	87.4
F2a	5	120	1.2729	3350	124.8
F2b	3	139	1.2994	3853	148
F2c	5	114	1.1511	2847	104.9
F2d	5	103	1.2575	2869	112.0

TABLE A5.2

BARLEY YIELD TRIAL COVARIATE SCORES

SAMPLE	A	B
REPLICATE 1		
S3	63	185
S2	48	195
S4	50	150
S1	59	124
F2	47	178
F3	50	226
F1	54	121
F4	53	110
REPLICATE 2		
F4	36	127
F2	47	153
F3	51	146
F1	52	111
S4	55	123
S3	40	83
S1	56	103
S2	41	95
REPLICATE 3		
F1	55	158
F4	45	138
F2	60	118
F3	90	132
S3	60	128
S2	38	90
S1	48	92
S4	54	107
REPLICATE 4		
S2	41	121
S4	50	104
S3	50	123
S1	43	99
F4	52	126
F1	53	103
F3	30	88
F2	51	126

A = NO SEEDLINGS / METRE ROW (COUNTED 30/10/85)
B = NO TILLERS / METRE ROW (COUNTED 2/3/86)

S = SF
F = FE

1 = 10 g / m2
2 = 20 g / m2
3 = 30 g / m2
4 = control

APPENDIX 6 WEATHER DATA

Table A6.1 Rainfall at Lincoln and Gore during trial seasons

Rainfall - Lincoln 1985 (mm)

Date	Aug.	Sep.	Oct.	Nov.
1	0.4	0.0	0.0	0.0
2	9.8	0.0	0.0	9.0
3	4.2	0.6	5.3	4.1
4	0.0	12.0	1.4	0.0
5	0.0	2.9	0.0	20.2
6	0.0	3.9	0.0	0.0
7	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0
9	0.0	0.0	0.5	0.0
10	5.5	7.9	0.0	0.0
11	3.5	0.0	0.0	0.0
12	7.6	0.0	0.0	9.0
13	6.0	0.0	9.0	0.0
14	0.0	0.0	0.0	0.0
15	0.0	0.5	1.3	0.0
16	0.0	0.0	0.0	
17	0.0	0.0	0.0	
18	0.0	0.0	0.0	
19	0.0	0.0	0.0	
20	0.0	0.0	0.5	
21	0.0	0.0	2.3	
22	0.0	0.0	0.0	
23	0.0	0.0	0.3	
24	0.0	0.0	0.0	
25	0.0	0.0	5.5	
26	8.0	1.3	5.2	
27	0.8	0.0	0.4	
28	0.0	0.0	0.0	
29	0.0	0.0	0.0	
30	0.0	0.0	0.0	
31	3.5		0.0	
Total rain	49.3	29.1	31.7	42.3
No wet days	10/31	3/30	11/31	4/15

Daily Rainfall - Gore 1985/86 (mm)

Day	Oct.	Nov.	Dec.	Jan.
1		7.5	9.7	10.6
2		2.7	1.1	1.1
3		13.5	1.2	0.0
4		4.4	0.2	0.0
5		1.2	0.0	0.0
6		0.0	0.0	
7		1.3	5.1	
8		0.0	0.7	
9		0.0	0.0	
10		0.0	0.0	
11		4.1	0.0	
12		2.2	0.0	
13		0.0	0.3	
14		0.0	1.3	
15		0.0	0.1	
16		0.1	0.0	
17		0.3	0.0	
18		0.5	0.0	
19		11.0	0.0	
20		3.2	0.0	
21		3.2	0.8	
22		1.2	1.1	
23		0.5	20.8	
24		0.0	0.1	
25		0.0	0.0	
26		0.0	3.3	
27		0.0	0.4	
28		0.1	11.9	
29	0.0	0.1	12.1	
30	0.0	6.5	1.1	
31	0.0		3.4	
TOTALS				
	0.0	63.6	74.7	11.7
NO WET DAYS				
	0/3	19/30	19/31	2/5

Rainfall Lincoln 1986/87 (mm)

DAY	JUNE	JULY	AUG.	SEP.	OCT.	NOV.	DEC.	JAN.
1		4.5	0.0	0.0	0.0	0.0	3.5	0.0
2		6.5	0.6	0.0	0.0	12.5	1.4	0.0
3		1.5	0.0	0.0	9.0	0.0	0.0	0.0
4		0.0	5.5	0.0	13.5	0.0	0.0	0.0
5		9.0	0.0	0.0	15.0	0.0	0.0	0.0
6		38.0	0.0	0.8	0.2	0.0	0.0	0.0
7		6.0	9.5	0.0	0.0	0.0	0.0	0.0
8		0.8	4.8	0.5	0.0	0.0	0.0	0.0
9		2.0	7.5	0.0	0.3	0.0	0.0	0.0
10		23.5	7.0	0.0	3.5	0.0	0.0	0.0
11		8.3	0.5	0.0	21.5	0.0	0.0	0.4
12		0.5	0.0	0.0	0.0	4.5	0.0	0.0
13		0.0	1.0	0.0	1.0	0.0	0.0	0.0
14		0.0	0.4	21.0	0.0	1.7	0.0	0.0
15		0.0	0.0	10.2	0.0	0.0	0.0	0.0
16		0.0	0.0	0.5	2.4	0.0	0.5	0.0
17		0.0	0.0	0.0	29.0	0.0	0.0	1.0
18		0.0	0.0	2.5	2.5	0.0	0.0	0.0
19		0.0	0.0	1.0	1.7	0.0	0.0	0.2
20	0.0	10.0	0.0	1.4	2.5	0.0	0.0	0.0
21	2.5	6.0	12.0	1.0	2.9	0.0	8.5	0.0
22	0.5	0.5	30.0	0.0	0.0	0.8	0.0	2.3
23	0.0	0.0	37.5	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.5	0.0	0.0	19.5	0.0	0.0
25	1.5	12.8	0.0	0.0	0.0	41.5	0.0	4.7
26	2.0	0.6	0.0	0.0	0.9	9.5	0.0	0.0
27	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0
28	0.0	0.0	5.3	10.5	0.0	1.2	0.0	
29	0.0	0.0	1.4	0.0	0.0	2.2	4.0	
30	7.1	0.0	0.5	0.0	0.0	0.9	18.3	
31		1.0	0.0		0.0		0.0	
TOTALS								
	13.6	131.5	124.0	49.4	105.9	94.3	36.2	8.6
NO WET DAYS								
	5/11	17/31	16/31	10/30	16/31	10/30	6/31	5/27

Table A6.2 Temperatures at Lincoln and Gore during trial seasons

Monthly temperatures (°C)

	T min.	T max.	T mean
GORE 1986/86			
Month			
Oct 29-31	6.8	19.4	13.1
Nov.	2.2	24.8	13.5
Dec. 1-18	3.2	26.1	14.65
1-21	3.2	27.0	15.1
Jan. 1-5	7.0	26.5	16.75
LINCOLN 1985			
Month			
Aug.	-3.6	19.6	8
Sep.	0.0	21.6	10.8
Oct.	-1.6	24.3	11.35
Nov. 1-10	3.5	23.3	13.4
1-15	3.5	26.1	14.8
LINCOLN 1986			
Month			
June 20-30	-1.0	15.0	7.0
July	-5.0	15.0	5.0
Aug.	-3.0	15.0	6.0
Sep.	-3.0	21.0	9.0
Oct.	2.0	22.0	12.0
Nov.	2.0	25.0	13.5
Dec.	5.0	26.0	15.5
Jan. 1-27	3.0	30.0	16.7

APPENDIX 7 - DIRECTIVE FILES FOR GENSTAT STATISTICS PACKAGE

TABLE A7.1

CHEMICAL FIELD SPRAYING TRIAL

```
'REFE' EYESPOT
'SCALAR' NUNITS = 64
'RUN'
'UNITS' $ NUNITS
'HEAD' H = ''  EYE-SPOT FIELD TRIAL...8 TREATMENTS...2 DENSITIES''
'NAMES' DN = LOW,HIGH
  : TN = BEN,BENSTIK,PRO,PROSTIK,BEN/PRO,B/PSTIK,B/P.5,CONTROL
'FACTOR' REP $ 4 = 16(4,3,2,1)
  : DENSITY $ DN  : TRT $ TN
'INPUT' 2
'READ/PRIN=Z,FLEV=F' TRT,DENSITY,PLDENS,SC(1...6)
'INPUT' 1
'CALC' NOSTEMS = VSUM(SC(1...6))
  : SCORE = 20*(SC(2)+2*SC(3)+3*SC(4)+4* SC(5)+5*SC(6))/NOSTEMS
'DESC' SCORE $;H
'TERMS/DVSET=F' SCORE+TRT+TRT.PLDENS
'Y' SCORE
'FIT/INT=N' TRT+TRT.PLDENS
'RUN'
'CLOSE'
'STOP'
```

TABLE A7.2

GROWTH ROOM CULTIVAR TRIAL - MAIN WHEAT AND TRITICALE TRIAL

```
      'REFE' GROWTHROOM
      'UNITS' $ 1296
      'NAME' CVNM = BOUNTY,TIRITEA,TAKAHE,OTANE,RONGOTEA,KAREREE,ARANUI,SALVO,
                    LASKO
        : FNM = CONTROL,SLOW,FAST
      'FACTOR' CV $ CVNM = 12(3,1,4,5,8,7,2,9,6,
                              8,9,1,3,4,7,6,2,5,
                              7,8,4,9,5,3,1,6,2,
                              7,8,4,9,5,3,1,6,2,
                              8,9,1,3,4,7,6,2,5,
                              3,1,4,5,8,7,2,9,6,
                              7,8,4,9,5,3,1,6,2,
                              8,9,1,3,4,7,6,2,5,
                              3,1,4,5,8,7,2,9,6,
                              7,8,4,9,5,3,1,6,2,
                              8,9,1,3,4,7,6,2,5,
                              3,1,4,5,8,7,2,9,6)
       :  FUNGI $FNM = 108(1,2,3,3,1,2,2,3,1,2,3,1)

       :  ISOL $ 3 = 108(1),(4(1,2,3))27,108(1),(4(1,2,3))27,
                     108(1),(4(1,2,3))18,108(1)
       : PLS $12 = (1...12)108
       : PLANTS $ 4 = (1...4)324
     : REPS $ 4 = 324(1...4)
     'INPUT' 2
     'READ' SCORE
     'INPUT' 1
'TABLE' TAB1 $ FUNGI,CV,ISOL,REPS
'TABULATE/PRIN=M,EMPTY=MV' SCORE ;MEANS=TAB1
'RUN'
'UNITS' $ 324
'FACTOR'CVS $ CVNM :REP $ 4 : FUN $ FNM : ISOLS $ 3
'GENE' FUN,CVS,ISOLS,REP
'EQUATE' MNSC= TAB1
'RESTRICT' MNSC $ FUN=2
'BLOCKS' REPS/CV/PLS
'TREAT' CVS*ISOLS
'ANOVA/PROB=Y' MNSC
'RESTRICT/C' MNSC $ FUN=3
'ANOVA/PROB=Y' MNSC
'RUN'
```

TABLE A7.3

GROWTH ROOM CULTIVAR TRIAL - MAIN BARLEY AND RYE TRIAL

```
'REFE' GROWTHROOM
'UNITS' $ 1152
'NAME' CVNM = GWYLAN,FLEET,TRIUMPH,KYM,GOLDSPEAR,RAPAKI,DOMINANT,RAHU
  : FNM = CONTROL,SLOW,FAST
'FACTOR' CV $ CVNM = 12(7,5,4,1,8,3,2,6,
                        2,3,6,5,7,4,1,8,
                        3,2,6,5,7,1,4,8,
                        2,3,6,5,7,4,1,8,
                        7,5,4,1,8,3,2,6,
                        3,2,6,5,7,1,4,8,
                        2,3,6,5,7,4,1,8,
                        3,2,6,5,7,1,4,8,
                        7,5,4,1,8,3,2,6,
                        3,2,6,5,7,1,4,8,
                        7,5,4,1,8,3,2,6,
                        2,3,6,5,7,4,1,8)

  :  FUNGI $FNM = 96(1,3,2,2,1,2,1,3,2,3,2,1)

  :  ISOL $ 3 = 96(1),(4(1,2,3))24,96(1),(4(1,2,3))8,96(1),
     (4(1,2,3))32,96(1)
: REPS $ 12 = 288(1...4)
'INPUT' 2
'READ' SCORE
'INPUT'1
'TABLE' TAB(1,2) $ FUNGI,CV,ISOL,REPS
'TABU/EMPTY=MV,PRIN=M' SCORE  ;MEANS=TAB(1)
'RUN'
'UNITS' $288
'FACTOR'CVS $CVNM  :REP $ 4 : FUN $ ISOLS  $ 3
'EQUATE' MNSC= TAB(1)
'RESTRICT' MNSC $ FUN=2
'BLOCKS' REPS/CV/ISOLS
'TREAT' CVS*ISOLS
'ANOVA/PROB=Y'MNSC
'RESTRICT/C' MNSC $ FUN=3
'ANOVA/PROB=Y' MNSC
'RUN'
```

TABLE A7.4

GROWTH ROOM CULTIVAR TRIAL - SLOW-FEATHERY ONLY WHEAT AND TRITICALE

```
      'REFE' GROWTHROOM
      'UNITS' $ 432
      'NAME' CVNM = BOUNTY,TIRITEA,TAKAHE,OTANE,RONGOTEA,KARERE,ARANUI,SALVO,
                    LASKO
      'FACTOR' CV $ CVNM = 12(8,9,1,3,4,7,6,2,5,
                              7,8,4,9,5,3,1,6,2,
                              3,1,4,5,8,7,2,9,6,
                              7,8,4,9,5,3,1,6,2)

       : ISOL $ 3 = (4(1,2,3))9,(4(1,2,3))9,(4(1,2,3))9,(4(1,2,3))9
       : PLANTS $ 4 = (1...4)108
       : REPS $ 4 = 108(1...4)
     'INPUT' 2
     'READ' SCORE
     'INPUT'1
    'TABLE' TAB1 $CV,ISOL,REPS
'TABU/EMPTY=MV' SCORE;MEANS=TAB1
'RUN'
'UNITS' $ 108
'FACTOR' CVS $  CVNM :REP $4 :ISO $3
'GENE' CVS,ISO,REP
'EQUATE' MNSCORE = TAB1
 'BLOCKS' REP/CVS/ISO
     'TREAT' CVS*ISO
     'ANOVA/PROB=Y' MNSCORE
     'RUN'
'CLOSE'
'STOP'
```

TABLE A7.5

GROWTH ROOM CULTIVAR TRIAL - SLOW-FEATHERY ONLY BARLEY AND RYE

```
      'REFE' GROWTHROOM
      'UNITS' $ 384
      'NAME' CVNM = GWYLAN,FLEET,TRIUMPH,KYM,GOLDSPEAR,RAPAKI,DOMINANT,RAHU

      'FACTOR' CV $ CVNM = 12(3,2,6,5,7,1,4,8,
                              2,3,6,5,7,4,1,8,
                              7,5,4,1,8,3,2,6,
                              2,3,6,5,7,4,1,8)

       : ISOL $ 3 = (4(1,2,3))32
       : PLANTS $ 4 = (1...4)96
       : REPS $ 4 = 96(1...4)
     'INPUT' 2
     'READ' SCORE
     'INPUT'1
    'TABLE' TAB1 $CV,ISOL,REPS
'TABU/EMPTY=MV,PRIN=AM' SCORE;MEANS=TAB1
'RUN'
'UNITS' $ 96
'FACTOR' CVS $  CVNM : REP $4 :ISO $3
'GENE' CVS,ISO,REP
'EQUATE' MNSCR = TAB1
 'BLOCKS' REP/CVS/ISO
     'TREAT' CVS*ISO
     'ANOVA/PROB=Y' MNSCR
     'RUN'
'CLOSE'
'STOP'
```

TABLE A7.6

LINCOLN CULTIVAR FIELD TRIAL

```
'REFE' FIELD
''
 FIELD TRIAL LINCOLN
''
'UNITS' $ 270
'NAME' CVNM = SALVO,LASKO,ARANUI,KARERE,RAPAKI,DOMINANT,RAHU,
              BOUNTY,TAKAHE,OTANE,TIRITEA,N8020,RONGOTEA,
              GWYLAN,TRIUMPH,FLEET,KYM,GOLDSPEAR
  : FNM = CONTROL,SLOW,FAST
'FACTOR' CV $ CVNM = 8,9,10,13,4,18,3,2,5,7,14,16,12,6,1,11,15,17,
                     8,17,16,18,15,10,3,11,14,6,7,9,1,2,12,13,5,4,
                     16,17,13,10,5,3,11,6,2,12,18,4,14,1,8,7,9,15,
                     8,17,16,18,15,10,3,11,14,6,7,9,1,2,12,13,5,4,
                     8,9,10,13,4,18,3,2,5,7,14,16,12,6,1,11,15,17,
                     3,11,13,5,14,16,1,18,7,10,12,15,2,6,4,8,17,9,
                     16,17,13,10,5,3,11,6,2,12,18,4,14,1,8,7,9,15,
                     3,11,13,5,14,16,1,18,7,10,12,15,2,6,4,8,17,9,
                     8,9,10,13,4,18,3,2,5,7,14,16,12,6,1,11,15,17,
                     3,11,13,5,14,16,1,18,7,10,12,15,2,6,4,8,17,9,
                     16,17,13,10,5,3,11,6,2,12,18,4,14,1,8,7,9,15,
                     8,17,16,18,15,10,3,11,14,6,7,9,1,2,12,13,5,4,
                     3,11,13,5,14,16,1,18,7,10,12,15,2,6,4,8,17,9,
                     16,17,13,10,5,3,11,6,2,12,18,4,14,1,8,7,9,15,
                     8,17,16,18,15,10,3,11,14,6,7,9,1,2,12,13,5,4
  :  FUNGI $FNM = 18(1,3,2,2,3,1,1,2,3,3,2,1,1,2,3)
  : REPS $ 5 = 54(1...5)
'INPUT' 2
'READ' SC(1...20)
'INPUT' 1
'CALC' SCORE =   VMEAN(SC(1...20))*100/8
'RESTRICT/C' SCORE$ FUNGI = 1
'BLOCKS' REPS/FUNGI/CV
'TREAT' FUNGI*CV
'ANOVA/PROB=Y' SCORE
'RUN'
'CLOSE'
'STOP'
```

TABLE A7.7

GORE CULTIVAR FIELD TRIAL

```
'REFE' FIELD
''
 FIELD TRIAL GORE
''
'UNITS' $ 288

'NAME' CVNM = SALVO,LASKO,ARANUI,KARERE,RAPAKI,DOMINANT,RAHU,
              BOUNTY,TAKAHE,OTANE,TIRITEA,N8020,RONGOTEA,
              GWYLAN,TRIUMPH,FLEET,KYM,GOLDSPEAR
  : FNM = CONTROL,SLOW,FAST
'FACTOR' CV $ CVNM = 3,11,13,5,14,16,1,18,7,10,12,15,2,6,4,8,17,9,
                     16,17,13,10,5,3,11,6,2,12,18,4,14,1,8,7,9,15,
                     8,17,16,18,15,10,3,11,14,6,7,9,1,2,12,13,5,4,
                     8,9,10,13,4,18,3,2,5,7,14,16,12,6,1,11,15,17,
                     16,17,13,10,5,3,11,6,2,12,18,4,14,1,8,7,9,15,
                     3,11,13,5,14,16,1,18,7,10,12,15,2,6,4,8,17,9,
                     8,9,10,13,4,18,3,2,5,7,14,16,12,6,1,11,15,17,
                     8,17,16,18,15,10,3,11,14,6,7,9,1,2,12,13,5,4,
                     8,17,16,18,15,10,3,11,14,6,7,9,1,2,12,13,5,4,
                     8,9,10,13,4,18,3,2,5,7,14,16,12,6,1,11,15,17,
                     3,11,13,5,14,16,1,18,7,10,12,15,2,6,4,8,17,9,
                     16,17,13,10,5,3,11,6,2,12,18,4,14,1,8,7,9,15,
                     8,9,10,13,4,18,3,2,5,7,14,16,12,6,1,11,15,17,
                     8,17,16,18,15,10,3,11,14,6,7,9,1,2,12,13,5,4,
                     16,17,13,10,5,3,11,6,2,12,18,4,14,1,8,7,9,15,
                     3,11,13,5,14,16,1,18,7,10,12,15,2,6,4,8,17,9

   : ROWS $ 4 = (18(1...4))4  : COLS $ 4 = 72(1...4)
   :  FUNGI $FNM = 18(3,2,1,3,1,3,2,1,2,1,3,2,3,2,1,3)
  : REP $ 6 = 18(1,1,1,5,2,2,2,5,3,3,3,5,4,4,4,6)
'TABL/M' TTT $ CV,FUNGI
'INPUT' 2
'READ/PRIN=Z' SC(1...20)
'INPUT' 1
'CALC' SCORE = VMEAN(SC(1...20))*100/8
'REST/C' SCORE $ REP,FUNGI = 6,1
'BLOCKS' REP/FUNGI/CV
'TREAT' FUNGI*CV
'FOR' XVAR = SCORE
'ANOVA/PROB=Y' XVAR
'REPE'
'RUN'
'CLOSE'
'STOP'
```

TABLE A7.8

BARLEY YIELD TRIAL

```
'REFERENCE' BARLY

'UNIT' $128

'NAME' FNM = SLOW,FAST
'NAME' RTNM = X,2X,3X,CONTROL

'FACTOR' BLOCK $4 = 32(1...4)
    :    FUNGI $FNM = 16(1,2,2,1,2,1,1,2)
    :    RATE  $RTNM = 4(3,2,4,1,2,3,1,4,4,2,3,1,4,3,1,2,
                         1,4,2,3,3,2,1,4,2,4,3,1,4,1,3,2)
    :   PLANT  $4 =  (1...4)32
'INPUT' 2
'READ' SCORE, NHEADS, MWTHD, NGRAIN, GRWT
'INPUT' 1

'CALC' MGRHD = NGRAIN/NHEADS
'CALC' MWGHD = GRWT/NHEADS
'CALC' THGWT = (GRWT/NGRAIN)*1000

'BLOCKS' BLOCK/FUNGI/RATE/PLANT
'TREAT' FUNGI*RATE
'ANOVA/PROB=Y' MWGHD, MGRHD, MWTHD
'RUN'
'CLOSE'
'STOP'
```

TABLEA7.9

BARLEY YIELD TRIAL COVARIANCE ANALYSIS

```
'REFERENCE' BARLY

'UNIT' $128

'NAME' FNM = SLOW,FAST
'NAME' RTNM = X,2X,3X,CONTROL

'FACTOR' BLOCK $4 = 32(1...4)
    :    FUNGI $FNM = 16(1,2,2,1,2,1,1,2)
    :    RATE  $RTNM = 4(3,2,4,1,2,3,1,4,4,2,3,1,4,3,1,2,
                         1,4,2,3,3,2,1,4,2,4,3,1,4,1,3,2)
    :   PLANT  $4 =  (1...4)32
'INPUT' 2
'READ' SCORE, NHEADS, MWTHD, NGRAIN, GRWT
'READ' BEF, AFT
'INPUT' 1

'CALC' MGRHD = NGRAIN/NHEADS
'CALC' MWGHD = GRWT/NHEADS
'CALC' THGWT = (GRWT/NGRAIN)*1000

'COVARIATE' BEF,AFT

'BLOCKS' BLOCK/FUNGI/RATE/PLANT
'TREAT' FUNGI*RATE
'ANOVA/PROB=Y' SCORE, MWTHD, MGRHD, MWGHD, THGWT
'RUN'
'CLOSE'
'STOP'
```